Vol. LXXIII, No. 2 October, 1937

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE DIURNAL MIGRATION OF DEEP-WATER ANIMALS

J. H. WELSH, F. A. CHACE, JR., AND R. F. NUNNEMACHER

(From the Woods Hole Oceanographic Institution[1] and the Biological Laboratories, Harvard University)

The diurnal vertical migration of planktonic organisms in the sea is a well-known phenomenon (Russell, 1927; Clarke, 1933; 1934).[2] Many more of the actively swimming animals, such as copepods, are found nearer the surface at night than during the day. For example, the level of maximum numbers of adult *Calanus*, in the Gulf of Maine, may be around 40 meters during the night while during the day it may be below 120 meters (Clarke, 1934). It is generally agreed that the most important external factor which regulates these daily movements is light. The greatest depth to which light may have an effect, however, is not known, since almost all studies have been confined to relatively shallow water. Obviously it would depend on the locality and the organisms being investigated, for an animal living at a considerable depth in a region where the water was very transparent might be influenced by the penetrating light, while another organism at the same depth in a region of low transparency would be unaffected. If diurnal migrations occur in deep water they must be considered in any study of vertical distribution. The lowest level at which they occur might be a satisfactory measure of the lower limit of the photic zone. These and other problems of the biology of the deeper water organisms have a direct relation to the possible vertical movements which may occur at levels deeper than any which have been adequately investigated. For these reasons a study was begun of diurnal migrations in deep water during a cruise of " Atlantis " to the Sargasso Sea in 1936.

During the cruise of the " Michael Sars " enough data were obtained from hauls with open nets to indicate that certain of the deep-water fishes and decapod crustaceans were to be found at higher levels during the night than during the day (Murray and Hjort, 1912). The " Michael Sars " data, however, did not indicate how deep such move-

[1] Contribution No. 145.

[2] A rather complete list of references may be found in these papers.

ments might occur, and are conclusive for only a few species of crustaceans and fishes.

The Sargasso Sea was selected for the present investigation since collections with closing nets were being made in this region, and because of the transparency of the water. Helland-Hansen (1931) had found that photographic plates exposed at a depth of 1,000 meters in waters between the Canary Islands and the Sargasso Sea were blackened after an exposure of 80 minutes. Clarke (1936) had calculated from data obtained by Clarke and Oster (1934) that there is sufficient light to enable deep sea fishes to see objects at a depth of 430 meters or deeper in the Sargasso Sea, depending on certain assumptions regarding their

TABLE I

Showing depths, time of day and duration of hauls made with closing nets at "Atlantis" Station 2667.

Haul	Depth	Date	Nets Open	Nets Closed
	meters			
1	400 800	Sept. 6	6:52 a.m.	7:52 a.m.
2	400 800	Sept. 7	4:07 a.m.	5:10 a.m.
3	400 800	Sept. 7	6:57 a.m.	7:57 a.m.
4	400 800	Sept. 7	9:31 a.m.	11:31 a.m.
5	400 800	Sept. 7	1:18 p.m.	3:18 p.m.
6	400 800	Sept. 7	5:10 p.m.	7:10 p.m.
7	400 800	Sept. 7	8:57 p.m.	10:57 p.m.
8	400	Sept. 8	12:39 a.m.	2.39 a.m.
	800	Sept. 8	Net attached improperly, failed to fish.	

visual acuity and spectral sensitivity. Light of an intensity sufficient to affect the general activity of an animal would be found below the level at which objects could be seen.

Since it was desirable to make as many hauls as possible in a twenty-four-hour period only two closing nets were used and they were set to tow at 400- and 800-meter levels. The location of the station (2667) was 35° 40′ N. and 69° 36′ W. The duration of each haul and the time of day may be seen in Table I. One haul was made on September 6 with the nets open at 6:52 A.M. and closed at 7:52 A.M. A rough sea prevented further use of the nets that day but tows were resumed on September 7. The first three hauls were each of one hour duration, but

the absence of salps and other forms which sometimes prevent longer tows, and the relatively small catches made it possible and desirable to increase the time of towing of subsequent hauls to two hours. In order to compare the catches the numbers of organisms taken in hauls 1, 2 and 3 have been doubled.

Since a modification of the closing nets developed and described by Leavitt (1935) were used for these hauls, and since they worked most successfully they will be briefly described and figured. The method of

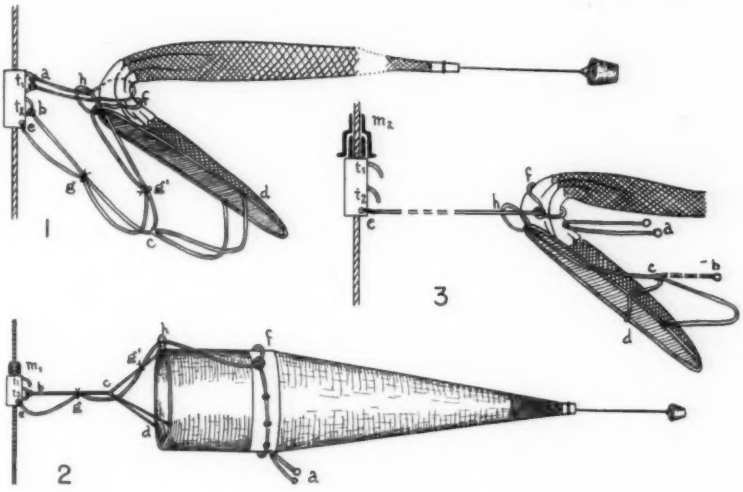


FIG. 1. Net closed, descending.
FIG. 2. Net open, towing.
FIG. 3. Net closed, ascending.

a. Fetter rope.
b. Single towing rope.
c. Three-point towing bridle.
d. Attachment of bridle on two-meter ring.
e. Attachment of pursing rope.
f. Pursing rope around belly-band of net.
g g'. Light string stops.
h. Small iron ring.
m_1. First messenger.
m_2. Second messenger.
t_1. Primary release.
t_2. Secondary release.

opening and closing was the chief feature of this net which differed from the one used by Leavitt. The releasing devices were the same. The changes were made by Mr. Nunnemacher with the advice of Dr. H. B. Bigelow.

The accompanying figures show a net: (1) closed, descending; (2) open, towing; (3) closed, ascending. The net itself has a diameter of 2 meters at the mouth and is laced to a strong galvanized iron ring of 2 meters diameter. The sides of the net are made of stramin (6 threads

per cm.) and are parallel for the first 2 meters and then sewed to a 60 centimeter wide, heavy canvas belly-band to which six 5-centimeter brass rings are fastened at equal distances over the lower half of the band. From the rear edge of the belly-band the net tapers from a 2-meter diameter to one of 25 centimeters over a distance of 7 meters. The end is finished off with another canvas band. Into the end of the net a silk net of corresponding taper is sewed so that it lines the last meter of the stramin and prevents excessive chafing of the catch.

On lowering the net (Fig. 1) a short fetter rope (*a*) fastened at its mid-point to the bottom of the belly-band tightly encircles the folded canvas, passes through the small iron ring (*h*) which is attached to the rim of the net and both ends of the fetter rope are hooked into the primary release. The releasing devices were those previously used by Leavitt (1935) and they proved entirely satisfactory. The loose towing and pursing ropes are "stopped" (loosely tied with cotton string) at points *g* and *g'* to make fouling impossible. After lowering the net to the desired depth, the primary release is opened by the first messenger and the fetter rope easily falls out of the way below the net. The net in opening merely unfolds, the loop of the pursing rope having been previously opened to the diameter of the net at the belly-band and then folded up with it. Figure 2 shows the net being towed by the strong three-point towing bridle and the pursing rope loosely surrounding the belly-band and "stopped" to the towing bridle. A bucket is also shown tied to the end of the net to prevent the tail from fouling in the eddies behind the closed net. After the required time of towing the second messenger releases the towing bridle which falls out of the way. The pursing rope, securely fastened to the cable, now takes the strain, constricts the net at the belly-band and effectively closes the net (Fig. 3).

At each haul an open net was attached 50 meters below the lower closing net. Since the level of maximum numbers of certain of the red prawns and blackfish is near 850 meters in this region of the Sargasso Sea, at least during the day, large catches were always taken by the open net and this material is being used in connection with other studies such as the eyes of deep sea crustaceans (Welsh and Chace, 1937). With the method we used to determine the depth at which a closing net fishes it is impossible to be accurate within about $\pm$ 100 meters at a depth of 800 meters. One can keep the level of the nets relatively constant by so controlling the speed of the ship as to maintain a constant wire angle. But it is not possible to know very accurately the level of fishing because of certain unknown forces which affect the catenary of the wire. We used the calculations made by Leavitt for setting the nets at 400- and 800-meter levels. These were based on the length of towing cable out, the

pull of the nets when open, the weight at the end of the towing cable and the wire angle at the surface.

After sorting the hauls it was evident that some forms had not been taken in sufficient numbers to allow any definite conclusion to be drawn regarding their behavior. Others have not yet been identified. But several of the prawns of the families Acanthephyridæ and Sergestidæ, one of the chætognaths, and the copepods and fishes were present in considerable numbers and the data for these will be presented. The copepods were present in such very large numbers in some hauls at the 400-meter level that their numbers were estimated in those hauls and no attempt was made to separate and identify the several species. Fishes at 800 meters were also present in large numbers and counts were made of these in each haul but again they were not separated into genera or species. In spite of dealing with mixed populations of copepods and fishes, the data readily convince one that the more abundant species behave in a consistent manner and that their movements are obviously related to day and night. A remarkable correlation in the numbers of copepods and fishes in the separate hauls at the 400-meter level will be discussed later.

In the graphs the total number of animals of a group such as copepods, or of a species, where separations into species were made, taken in any haul is shown by a vertical bar. Haul 1 made on September 6 from 6:52 A.M. to 7:52 A.M. serves as a check on the consistency of catches from day to day as another haul was made on September 7 from 6:57 A.M. to 7:57 A.M. The lined bars represent the September 6 catches. When no representatives of a given species were present the word "none" appears in place of a bar. The net at the 800-meter level at the last haul made on September 8 from 12:39 A.M. to 2:39 A.M. failed to work as it was improperly attached; hence no data are available for this time and depth. The time of sunrise was 5:37 A.M., of sunset 6:19 P.M. at the position of this station on September 6. A heavy line at the bottom of each graph indicates the hours between sunset and sunrise, but it should be remembered that at any considerable depth the period of relative darkness would be longer than at the surface (Clarke, 1934). It should also be noted that haul number 6 began before sunset and continued some time afterward. Further remarks concerning a particular species or group of animals may best be made under separate headings.

Acanthephyridæ (Fig. 4)

The complete absence of any of the large red prawns from hauls made during the day at 400 meters was the first reassuring evidence that our results would add more convincing weight to the conclusions reached

by Murray and Hjort (1912). *Systellaspis debilis* and *Acanthephyra purpurea* had been taken previously in this region of the Sargasso Sea (Welsh and Chace, 1937) but in these earlier collections with closing

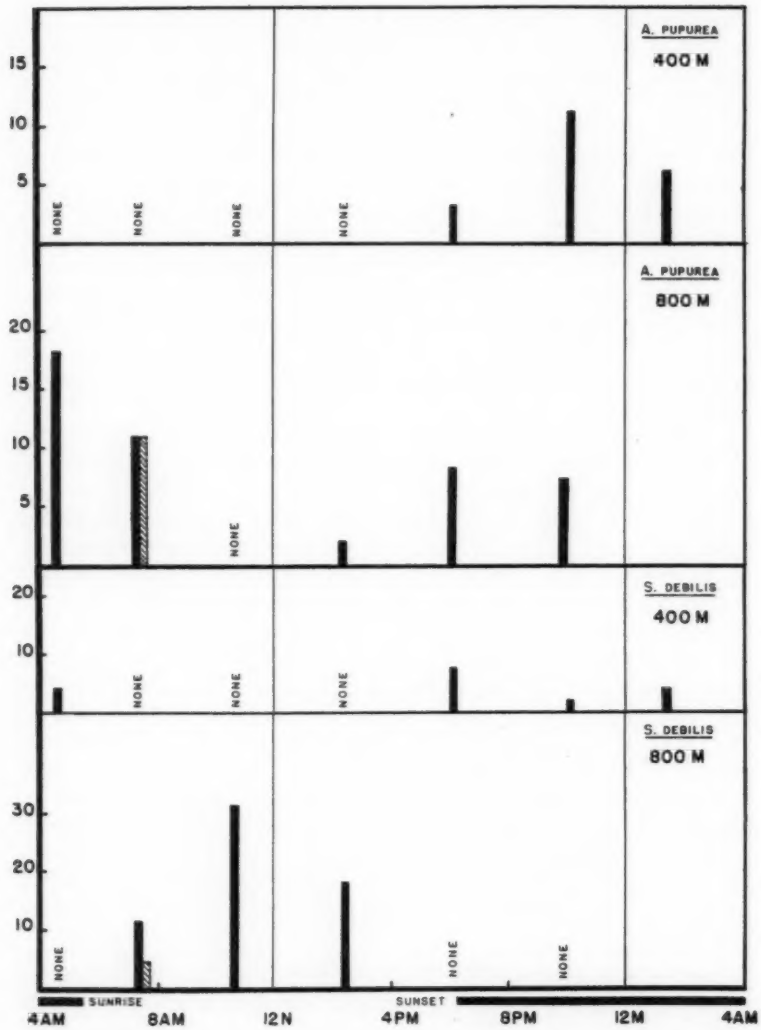


FIG. 4.

nets little attention had been paid to the time of day when the hauls were being made. At 800 meters the largest catch of *A. purpurea* was made in the early morning before sunrise, none were taken in the haul made nearest midday. On the other hand, *S. debilis* was taken at 800 meters only during daylight hours. Since it is known that *S. debilis* occurs at a somewhat higher level than *A. purpurea* a comparison of the catches of these two forms at the two levels strongly suggests that during the day the majority of *S. debilis* descend to a depth around 800 meters and the majority of *A. purpurea* to a somewhat greater depth.

Both *A. purpurea* and *S. debilis* have well-developed eyes. In contrast another acanthephyrid, *Hymenodora glacialis*, with degenerate eyes, has never been taken with closing nets from "Atlantis" at levels higher than 1,000 meters. It is an example of a form which has become adapted to a region below that at which penetrating sunlight has an effect on behavior and the development of the eyes. The upper limit of its distribution suggests that 1,000 meters is approximately the depth to which a biologically significant amount of sunlight can penetrate in water as transparent as that of the Sargasso Sea.

Sergestidæ (Fig. 5)

Of the sixteen species of *Sergestes* recorded from the North Atlantic, thirteen were taken at Station 2667. Of these thirteen, three species were taken in numbers large enough for statistical study. *Sergestes sargassi* appeared only in hauls at the 400-meter level and then only in those made during daylight hours. It was most numerous in the hauls made soon after sunrise. As this is known to be a comparatively shallow water species, it appears that it moves toward the surface during the night and returns to a depth around 400 meters during the day.

S. atlanticus was taken at the 400-meter level in two night hauls and in the haul which extended from about an hour before sunset until an hour after. None were taken at this level during the day. For some reason no sergestids of any species were taken in haul 7 at the 400-meter level in the late evening. At 800 meters *S. atlanticus* appeared in considerable numbers in the hauls made during the day, but except for one specimen which was taken in haul 2, none appeared in the other night hauls. Evidently this species migrates to deeper water during the day than does *S. sargassi*. It should be added that *S. atlanticus* has been taken in surface hauls made during the night.

The catches of *S. corniculum* at 400 meters were very much like those of *S. atlanticus* as regards relative numbers and times of appearance. At 800 meters, however, *S. corniculum* was found in relatively large numbers in all hauls during both day and night. It would appear

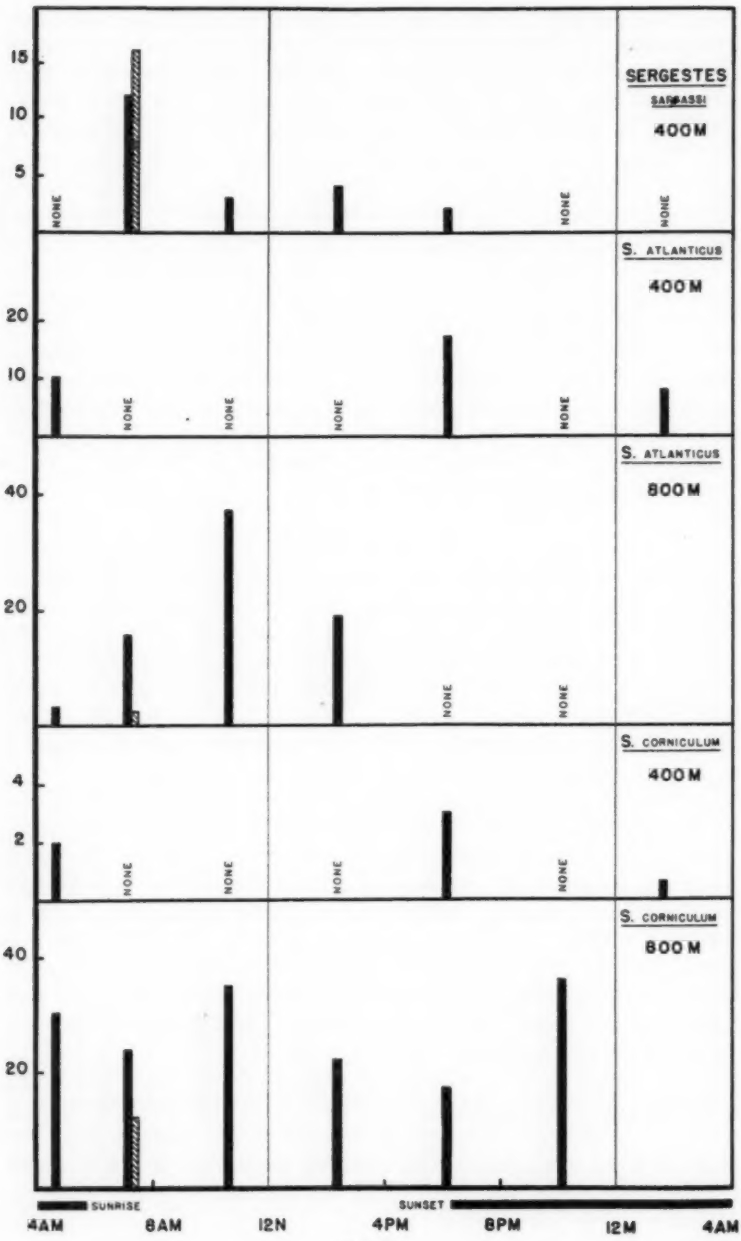

SERGESTES
SARGASSI
400M
S. ATLANTICUS
400M
S. ATLANTICUS
800M
S. CORNICULUM
400M
S. CORNICULUM
800M
NONE
SUNRISE
SUNSET
4AM
8AM
12N
4PM
8PM
12M
4AM

FIG. 5.

that it was a form that migrated less extensively than some of the other species of *Sergestes*. That there are sergestids which are found below a level to which a sufficient amount of light penetrates to cause any diurnal vertical movements is known from our records on *S. mollis*. This species has been taken with closing nets only at depths of 1,600 meters and greater. Thus we see in this series of sergestids that the level of maximum numbers depends on the species and the time of day or night; also that the average depth at which a given species may be found depends on the extent of daily migration, if it occurs at all.

Copepods and Fishes (Fig. 6)

Because of the number and variety of fishes they have not been separated into species and neither have the copepods. These two distinctly different types of animals show, at the 400-meter level, a very similar type of distribution at different times of day and night, and this correlation may be seen if the graphs in Fig. 6 are examined. The largest catches were taken at the 400-meter level in the haul made just before sunrise and the one which extended from about an hour before until an hour after sunset. Most of the other catches when compared with these were small. It is probable that the large catches were taken as the migrating copepods and fishes passed the 400-meter level on their ascent and descent. The very large catches before sunrise must mean that these animals anticipate the return of dawn and start downward before there is any increase in light intensity. When the numbers of fishes taken at 400 and 800 meters are compared it is first obvious that many more were taken at the deeper level, and then that many more were taken during the day than during the night. It is probable that this depth is not very far from the daytime level of maximum numbers of deep-water fishes of this region. The striking correlation in numbers of copepods and fishes at the 400-meter level suggests that the fishes move with the copepods and thereby stay near their chief food supply.

Sagitta hexaptera (Fig. 7)

One large, easily recognized chætognath, *Sagitta hexaptera*,[3] was separated and counted and will serve as another example of a form which was taken only at 400 meters. On examination of the graph of the distribution of this form it is seen that the largest catches were made near midday, the smallest in the late afternoon and early evening. It would seem that the level of maximum numbers of this species was in

[3] Identified by Miss Alice Beale.

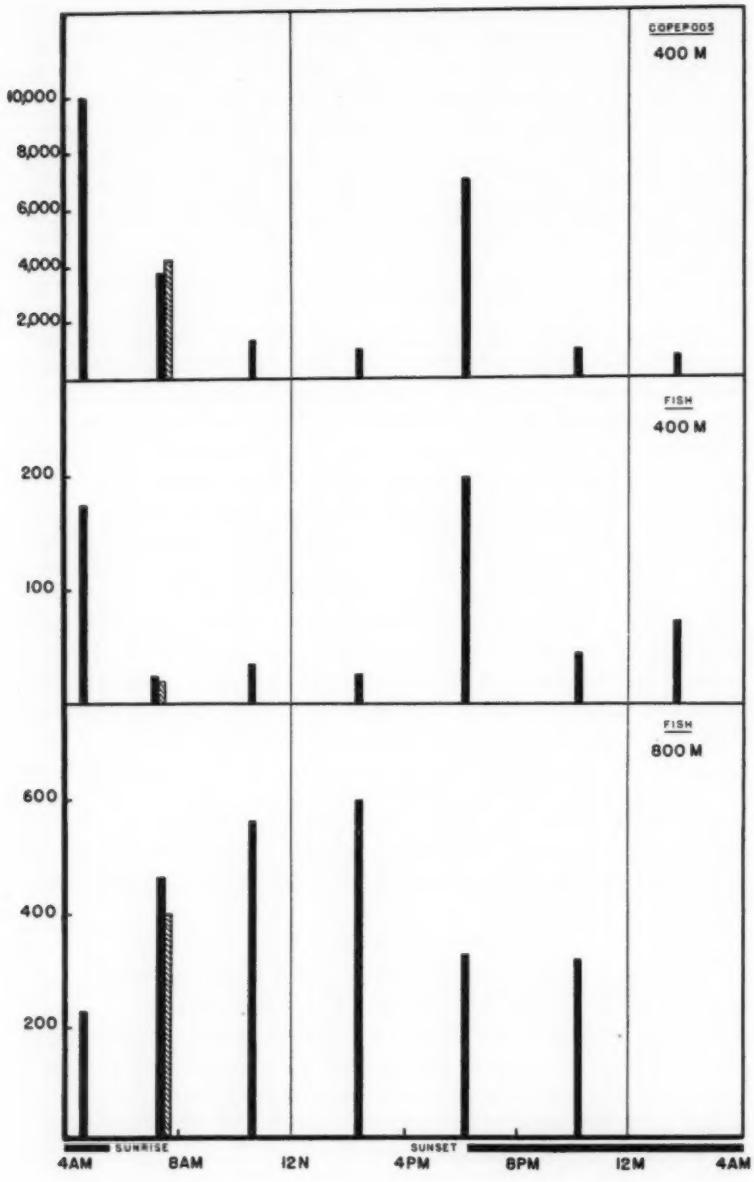

COPEPODS
400 M
10,000
8,000
6,000
4,000
2,000
FISH
400 M
200
100
FISH
800 M
600
400
200
SUNRISE
SUNSET
4AM
8AM
12N
4PM
8PM
12M
4AM

FIG. 6.

water shallower than 400 meters during the night and that they descended to near this level during the day.

Day to night differences in the numbers of certain species and groups of organisms, at depths of 400 and 800 meters, indicate not only that the animals of the deeper water of the Sargasso Sea migrate as do those of the shallow water, but that for certain species these daily migrations may be over a considerable distance. Light is probably the only day to night variable at a depth of 800 meters, or approximately one half mile; hence differences in light intensity must be largely responsible for these movements. There is, however, some indication that deep-water animals anticipate sunrise as Clarke (1934) found certain shallow water forms such as *Metridia* to do. This suggests that a physiological rhythm may

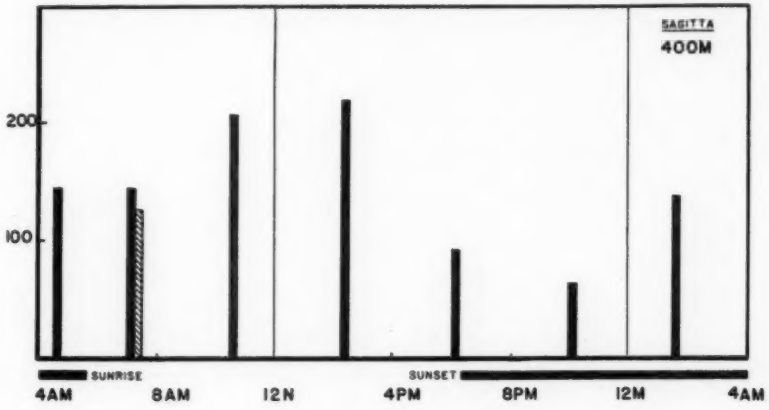


FIG. 7.

in part be responsible for their behavior. Diurnal rhythms are known to persist for considerable lengths of time in the absence of regularly recurring changes in light intensity (Welsh, 1936). An internal cycle resulting in periodic changes in activity would account for the downward movement which begins before there is an increase in light intensity.

Many questions have arisen which can be answered only by further investigations. It will be of interest to determine the greatest depth at which daily migrations occur and to correlate these movements with measurements of light intensity. It will be necessary to make collections at many more levels before final conclusions can be drawn regarding the behavior of the organisms discussed in this paper, and of the many which have been omitted from this discussion. Possible differences in

the behavior of animals with and without light organs should be investigated. The lower limit of the penetration of light of an intensity significant in determining the vertical distribution of animals should be correlated with the rather marked changes in temperature, salinity, oxygen and phosphates which occur at depths near 1,000 meters in certain parts of the Sargasso Sea. A combination of factors is doubtless responsible for keeping the majority of the macroplankton above 1,000 meters in this region, but it would be of interest to know which one, if any, is most important.

The collection and analysis of data of this kind is inevitably slow, but the vertical movement of organisms in the sea is of practical as well as of theoretical importance since the nightly aggregation of organisms near the surface increases greatly the potential food supply of surface fishes. Further work in the Sargasso Sea is planned and it should be possible to obtain a much more complete idea of the biology of deep-water organisms than we now possess.

LITERATURE CITED

CLARKE, G. L., 1933. Diurnal migration of plankton in the Gulf of Maine and its correlation with changes in submarine irradiation. *Biol. Bull.*, **65**: 402.

CLARKE, G. L., 1934. Further observations on the diurnal migration of copepods in the Gulf of Maine. *Biol. Bull.*, **67**: 432.

CLARKE, G. L., 1936. On the depth at which fish can see. *Ecology*, **17**: 452.

CLARKE, G. L., AND R. H. OSTER, 1934. The penetration of the blue and red components of daylight into Atlantic coastal waters and its relation to phytoplankton metabolism. *Biol. Bull.*, **67**: 59.

HELLAND-HANSEN, B., 1931. Physical oceanography and meteorology. *Rept. Michael Sars N. Atlantic Deep-sea Exp.*, 1910, **1**: 43.

LEAVITT, B. B., 1935. A quantitative study of the vertical distribution of the larger zoöplankton in deep water. *Biol. Bull.*, **68**: 115.

MURRAY, J., AND J. HJORT, 1912. The Depths of the Ocean. Macmillan and Co.

RUSSELL, F. S., 1927. The vertical distribution of plankton in the sea. *Biol. Rev.*, **2**: 213.

WELSH, J. H., 1936. Diurnal movements of the eye pigments of Anchistioides. *Biol. Bull.*, **70**: 217.

WELSH, J. H., AND F. A. CHACE, JR., 1937. Eyes of deep-sea crustaceans. I. Acanthephyridæ. *Biol. Bull.*, **72**: 57.

THE PROBLEM OF A PHYSIOLOGICAL GRADIENT IN MNEMIOPSIS DURING REGENERATION[1]

B. R. COONFIELD AND A. GOLDIN

(From the Department of Biology, Brooklyn College, and the Marine Biological Laboratory, Woods Hole, Massachusetts)

Experiments on certain ctenophores have shown the presence of physiological gradients in these animals. According to Child (1933) these animals show a decreasing susceptibility to toxic agents from the aboral to the oral end of a plate row. There are also definite indications that *Mnemiopsis leidyi* exhibits an aboral dominance in certain aspects of its activity (Coonfield, 1934 and 1936*a*). In addition to these reactions, it is known that this animal will regenerate readily (Coonfield, 1936*b*). All of these evidences suggested the advisability of making certain experiments on *Mnemiopsis leidyi* to test for a physiological gradient during regeneration and in response to grafts.

The experiments reported herein were of three types. In one, the time of regeneration following the removal of the aboral zone was recorded; in another, the time of regeneration in the plate rows was observed; and, in a third experiment, the reactions of *Mnemiopsis* to grafting of an apical organ was followed. Similar experiments have been used previously by Watanabe (1935) on *Euplanaria dorotocephala* and by Child (1935) on *Corymorpha*. The method of keeping *Mnemiopsis* during the experiments and of observing these animals was the same as reported by Coonfield (1936*b*). The temperature of the sea water in which the experimental animals were immersed ranged from 19.0° to 20.5° C. Each type of experiment is described and the results are given according to the headings: regenerating oral pieces, regenerating rows, and absorption of apical organ grafts.

Regenerating Oral Pieces

The purpose of these experiments was to record the time at which oral pieces of *Mnemiopsis* would regenerate organs which had been removed by a single cut across the body. These cuts were made at four levels: *A*, *B*, *C*, and *D* (Fig. 1). By these transverse cuts certain organs (Fig. 1) were removed along with the aboral portion of the body. We have assumed that regeneration was complete just as soon

[1] Contribution No. 23 from the Department of Biology, Brooklyn College.

as the lost organs had been reformed. At this time the experimental animals had not regained their former size, but we believe that only growth is necessary for this to be accomplished. The results of these experiments are shown in Table I.

Regenerating Rows

In the experiments reported here, the time required by rows to regenerate removed sections was recorded. A section including ten plates was removed from each of the four adtentacular rows at four different levels of the body. Level 1 begins at the apical organ and extends aborally; level 2 begins at the apical organ and extends orally; level 3 begins at the oral end of level 2 and extends orally; and level 4 begins at the oral end of level 3 and extends orally. Levels 1 and 2 were removed from the left and right rows respectively on one side, while levels 3 and 4 were removed from the left and right rows respectively on the other side of the animal. The healing and regenerating

Table I

Time in days required for aboral regeneration

Levels	Number operations	Number survived	Number regenerated	Average time
A	40	36	36	4.0
B	30	28	28	3.0
C	55	39	39	3.0
D	59	46	46	3.7

processes which followed the operations have been described adequately by Coonfield (1937*a*). The results of these experiments are shown in Table II.

Absorption of Apical Organ Grafts

In previous experiments (Coonfield, 1936*a*), it was found that apical organs which had been grafted to the surface of *Mnemiopsis* became absorbed by the host within a few days. The response of the host in these experiments suggested that this animal might be examined for a gradient by transplanting an apical organ to its body at different levels and observing the times at which the grafts are absorbed. Therefore a piece of an animal containing an apical organ, a portion of the infundibular funnel, and a part of the adradial canals was transplanted to the surface of another animal between its adtentacular rows. The levels are shown in Table III as 1, 2, and 3. Level 1 is just above the

apical organ; level 2 is mid-way between the apical organ and the bases of the auricles; and level 3 is near the bases of the auricles. Usually these grafts established connections with certain canals of the host as described by Coonfield (1937*b*). In these the order of connections was with the adtentacular, the tentacular, the paragastric canals, and with the stomodeum (Fig. 2, *A*). In some, however, this order in connecting

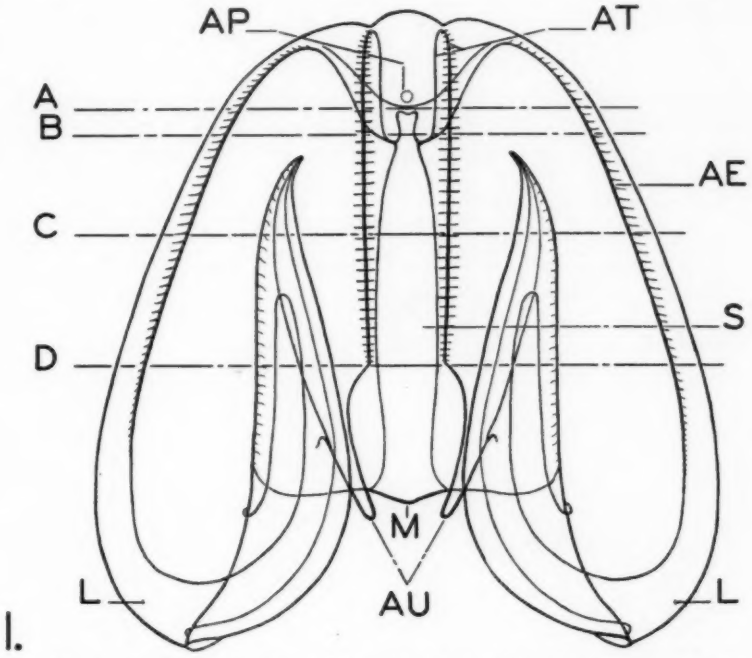


FIG. 1. This is a diagram of *Mnemiopsis* showing levels *A*, *B*, *C*, and *D* at which cross cuts were made. The organs as shown are: *AE*, adesophageal row; *AP*, apical organ; *AT*, adtentacular row; *AU*, auricles; *L*, lobes; *M*, mouth; *S*, stomodeum.

was not followed (Fig. 2, *B*). The results of these experiments are shown in Table III.

Since the apical organ is believed to exert a dominant influence over the other parts of the body of *Mnemiopsis* (Coonfield, 1936*a*), it is possible that this organ might be chiefly responsible for the absorption of the graft and would interfere with the detection of a physiological gradient. Therefore the aboral region including the apical organ was cut from specimens at the time that the transplantations were made.

TABLE II

Time in hours required for regeneration

Levels	Number operations	Number survived	Solid cord	Hollow canal	New plates
1	20	20	2.3	5.0	42.2
2	20	20	2.3	5.6	40.1
3	20	20	2.35	5.8	42.6
4	20	20	2.35	5.6	43.7

Controls were observed to detect any variation within groups of specimens. The results of the experiments are shown in Table IV. The levels indicated in this table correspond to the similar ones shown in Table III.

TABLE III

Time in days required for absorption of apical organ grafts. (The host was intact except for the transplantation.)

Levels	Number operations	Number survived	Number absorbed	Average time
1	17	8	8	9.25
2	17	14	14	12.25
3	16	8	8	10.50

TABLE IV

Time in days required for absorption of apical organ grafts. (Apical organ of the host was removed at time of transplantation.)

Level	Number operations	Regeneration		No regeneration		No absorption		No absorption No regeneration	
		Number absorbed	Average time	Number absorbed	Average time	Number regenerated	Average Time	Number	Time
2	24	7	11.6	5	11.4	2	10	10	7.1
3	15	2	12.0	2	9.0	3	6	8	8.1

DISCUSSION

Mnemiopsis will regenerate organs that have been removed and many of the changes taking place during regeneration can be observed easily (Coonfield, 1936*b*). Should a gradient be present during regeneration, there should be a difference in the time at which organs will

regenerate. In making this test specimens were cut across at four levels and the regenerating oral pieces were observed. The progressive changes following these cuttings at these levels were very similar. This resemblance is illustrated in Figs. 3 to 10 where the arrows which point to a plate in a similar location on each of a series show that it is possible to believe that in Figs. 3 to 6 are represented four different stages of one animal. These figures represent four different animals at four different stages during regeneration. In Figs. 7 to 10 four other specimens which were cut at a lower level than those in Figs. 3 to 6 are

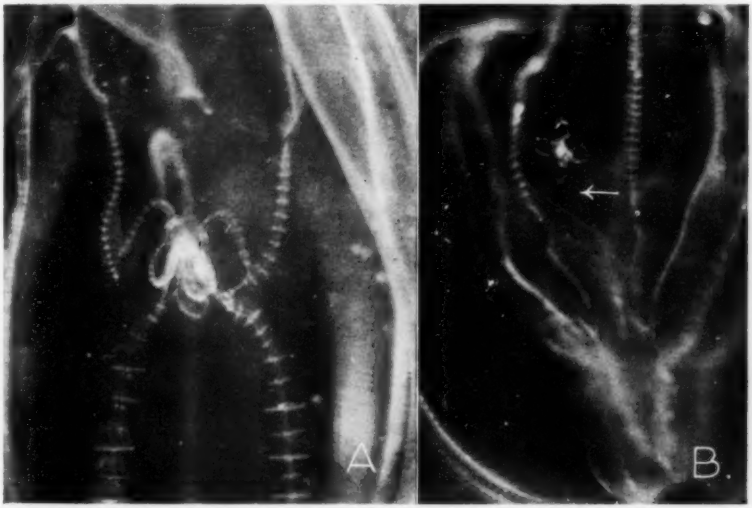


FIG. 2. These are photographs of the adtentacular surfaces of two specimens to which apical organs were grafted at level 3 (Table III). *A* shows the graft connected to two adtentacular canals of the host. *B* shows the graft connected to the tentacular canal (at arrow) of the host. Both photographs were taken five days after the operations.

shown. The results of these experiments (Table I) show clearly that there is no difference in the time of regeneration at the four levels of the body.

Following the removal of sections from plate rows the remaining ends of the rows pulled together, fused, stretched, and formed solid cords which later became hollow as described by Coonfield (1937*a*). Later new plates formed in the healed region and in between the old plates which had been pulled apart by the stretching. The results of these experiments are shown in Table II. Here the average time in

hours required for the formation of the solid cord of cells, the hollow canal, and the new plates is shown. There was no significant difference in the time of formation of these structures at the four levels of the body. This experiment is significant since the same organs of an individual were regenerating at the same time and at different levels. Furthermore there was no appreciable variation in the time of regeneration of these parts of rows when the results of experiments on individual animals were compared with each other.

Two methods of testing for a gradient by the absorption of apical organ grafts were employed. In one where the specimens were intact, only the time of absorption was involved. In the other where the apical organ of the host was removed at the time of the transplantation, the possible influence of this organ on the absorption of the graft was eliminated. The results obtained by the first method show that the grafts near the apical organ were absorbed at a slightly shorter time than those which were farther away from this organ. The difference in the time, however, is not enough to be significant. In those animals from which the apical organs had been removed at the time of the grafting (Table IV) the results show that there is no appreciable difference in the time of absorption of the grafts at different levels. It is clear also that a lower percentage of grafts was absorbed when the influence of the apical organ was removed. We believe that this low percentage of absorption was due to the absence of the influence of the apical organ. This influence is demonstrated by the delayed regeneration of an apical organ in those specimens on which the grafts persisted. We point out that in a relatively high percentage of these specimens the grafts persisted and there was no regeneration. We believe that the failure to regenerate was due to the inhibitory influence of the graft. In these specimens the grafts joined the various canals exactly as a normal apical organ is associated with the canals of the animal.

We conclude from the results of these experiments that a physiological gradient that has been shown to influence the times of regeneration

---

EXPLANATION OF PLATE I

FIGS. 3, 4, 5, and 6 are photographs of four specimens regenerating. They were all cut across at the same time and at level *A* (Fig. 1). They were photographed at 13, 39, 48, and 90 hours respectively after the apical ends had been removed. The arrows are pointing at similar plates on the four specimens to show the similarity of the regenerating processes.

FIGS. 7, 8, 9, and 10 are photographs of four specimens regenerating. They were cut across at the same time and at level *B* (Fig. 1). They were photographed at 13, 39, 48, and 90 hours respectively after the apical ends had been removed.

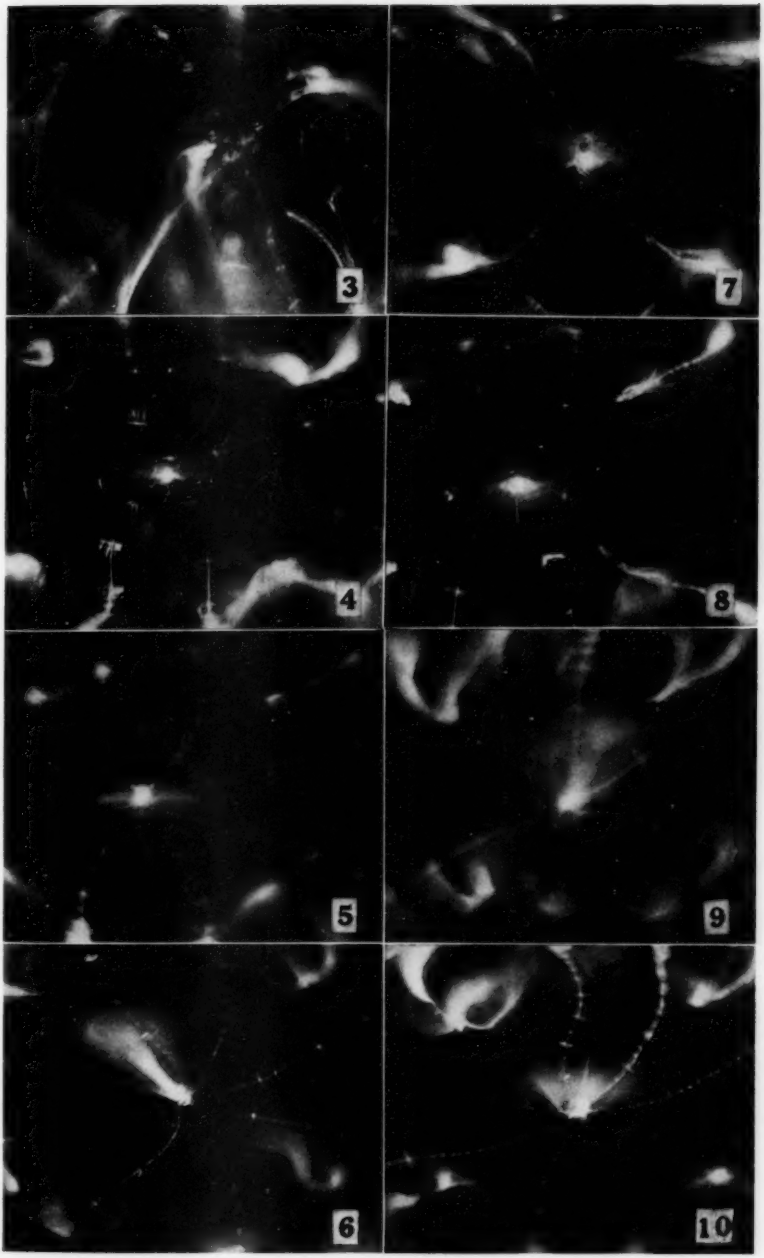


PLATE I

and the absorption of organs in other animals is not present in *Mnemiopsis leidyi*.

Conclusions

1. A physiological gradient which would influence the times of regeneration and absorption of grafts is not present in *Mnemiopsis*.

2. The time of regeneration of oral pieces following transverse cuts at four levels of the body is the same at all of these levels.

3. The time of regeneration following the extirpation of sections of plate rows at four levels of the body is the same at all of these levels.

4. The time of absorption of apical organ grafts is the same at three different levels of the body.

5. Retained apical organ grafts either inhibit or delay the regeneration of an apical organ by the host.

LITERATURE CITED

Child, C. M., 1933. The swimming plate rows of the ctenophore, Pleurobrachia, as gradients: with comparative data on other forms. *Jour. Compar. Neurol.*, **57**: 199.

Child, C. M., 1935. Dominance of hydranths induced by grafts in Corymorpha. *Jour. Exper. Zoöl.*, **71**: 375.

Coonfield, B. R., 1934. Coördination and movement of the swimming-plates of Mnemiopsis leidyi, Agassiz. *Biol. Bull.*, **66**: 10.

Coonfield, B. R., 1936*a*. Apical dominance and polarity in Mnemiopsis leidyi, Agassiz. *Biol. Bull.*, **70**: 460.

Coonfield, B. R., 1936*b*. Regeneration in Mnemiopsis leidyi, Agassiz. *Biol. Bull.*, **71**: 421.

Coonfield, B. R., 1937*a*. The regeneration of plate rows in Mnemiopsis leidyi, Agassiz. *Proc. Nat. Acad. Sci.*, **23**: 152.

Coonfield, B. R., 1937*b*. Symmetry and regulation in Mnemiopsis leidyi, Agassiz. *Biol. Bull.*, **72**: 299.

Watanabe, Y., 1935. Rate of head development as indicated by time of appearance of eyes in the reconstitution of Euplanaria dorotocephala. *Physiol. Zoöl.*, **8**: 41.

THE HEXOCTAHEDRON AND GROWTH [1]

OTTO GLASER AND GEORGE P. CHILD

(From the Biological Laboratories of Amherst College)

I

Kelvin's (1887) analysis of the conditions under which soap bubbles assume hexoctahedral form and together incorporate space without remnant, applies to tissue cells whenever these are surrounded and compressed on all sides by units essentially similar to themselves (Lewis, 1923, 1926, 1933). In all cases the more generalized the tissue and the more exact its quantification, the more closely the cell approximates a solid whose thirty-six equal edges bound eight hexagons and six non-contiguous squares.

II

Since cells and nuclei contain emulsions, we may anticipate, under appropriate conditions, either orthic or rhombic hexoctahedra among various kinds of protoplasmic inclusions. Indeed, Lewis (1926, p. 23) has verified Pouchet's (1847) discovery that polyhedra of this type occur among the yolk granules of a hardboiled egg. Here, despite much fortuity, we find practical realizations of the ideal form shown in Fig. 1. As Lewis has not depicted these polyhedra and Pouchet's figures are not easily available, a selection of coagulated yolk granules is reproduced in Fig. 2.

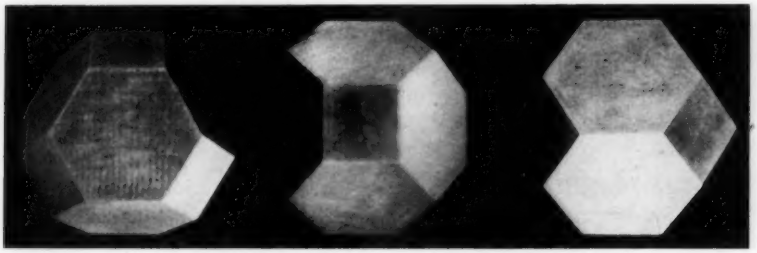
Comparable shapes, sometimes indistinguishable from orthic hexoctahedra, can be observed in dried cornstarch (Fig. 3). Many illustrations in Reichert's (1913) monograph indicate the same form, more or less modified, in other types of starch.

III

Translucent polyhedral bodies fill and often burst the nuclei in various tissues of "wilted" caterpillars (Fig. 4). In any given nucleus

[1] We are indebted to Professor George W. Bain of Amherst College for photographs of the polyhedral bodies of the silkworm. Dr. Bain first drew our attention to the fact that these bodies do not polarize light. We wish to express our appreciation also to Dr. R. W. Glaser of the Rockefeller Institute for material sent in 1936 and for the privilege of studying the photographic plates made in connection with the original Glaser, and Glaser and Chapman investigations on the polyhedral bodies of insects.

the polyhedra are remarkably uniform in size; in different nuclei they range from 0.5 to 15 μ in diameter (Glaser and Chapman, 1916).



A B C

FIG. 1. Models of the hexoctahedron. In the originals, made of aluminum, the edges are exactly 2 cm. in length. *A*, with hexagonal facet toward the observer and lying on the hexagon, opposite. *B*, with square facet toward the observer and lying on the square opposite. *C*, interhexagonal edge or dihedral angle toward the observer and resting on the interhexagonal edge opposite.

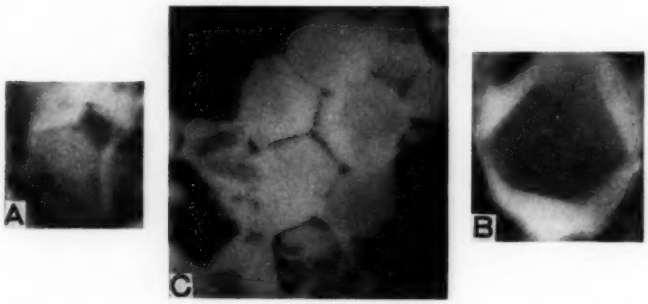


FIG. 2. Granules of coagulated egg-yolk under various illuminations and magnifications. No granule is completely in focus throughout.

A, B. Quadrilateral facets produced by truncation insufficient to realize equilateral edges.

C. A group of granules sufficiently uniform to illustrate practically ideal stacking. Focused on the planes of contact. The three dots are "waste" space.

Ocular recognition of form in three dimensions depends on optical sections combined with significant surface views. For the interpretation of these, oriented models (Fig. 1) are indispensable.[2]

[2] Useful models can be constructed after the patterns of Matzke (1931). Matzke has also published a careful analysis of the orthic tetrakaidecahedron and its aggregation without waste of space (1927).

Experimentation shows that hexoctahedra, in air, are most stable when resting on their hexagonal faces. In this position, the surface nearest the observer is also hexagonal and between this proximal and the basal hexagon are two alternating tiers in each of which three lateral hexagons alternate with three squares (Fig. 1, *A*). In a second position, most frequent in viscous liquids, the polyhedron is supported by the overhanging edges of the lateral hexagons and comes to rest on a square face. Now the uppermost surface is also square and separated from its basal counterpart by two tiers of four hexagons each and an equatorial tier of four non-contiguous squares (Fig. 1, *B*).

To interpret microscopic polyhedra requires patience. Even though the facets are true planes, we cannot assume that every individual resting on a square or hexagon is also level. A specific polyhedron may deviate

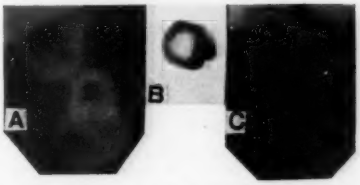


FIG. 3. Granules of dried cornstarch photographed by reflected light under various magnifications.

A. Upper left granule showing a clearly quadrilateral face; lower right, showing square facet, vaguely.

B. Lateral view of cornstarch granule showing two hexagonal facets; the lower one with a glitter point. To the right a quadrilateral face.

C. Similar to *B* but showing hila in hexagonal facets.

from the ideal, or have its basal facet tilted by neighbors or fragments of foreign material. Thus, an optical section may not conform with expectations based on simple square or hexagonal orientation.

What then is required to establish the hexoctahedral form? Clearly, the object must be three-dimensional; must possess facets; and these, when not hexagonal, must be square. Squares cannot be contiguous. Facial angles, when not 120°, must be 90° and all edges must be equal. We should expect sections in which the hexagons are not equilateral and sections indicative of more than six sides. Octagons, decagons and dodecagons are all possible. Fragmentary outlines of the higher polygons are not easily intelligible.

Photographs, based on the two stable orientations of the models in Fig. 1 and including only samples of the typical surface views and optical sections most frequently encountered with the polyhedral bodies

of the silk worm, are shown in Fig. 5. These records enable us to postulate the hexoctahedral form for the polyhedral bodies of "wilt."

If sufficiently familiar with the form, the observer can recognize the three-dimensional character of the polyhedra and indications of the two types of facet even at the lower magnifications shown in Fig. 4. To these views, we add the evidence secured by dark field and unilateral illumination. In favorable instances such lighting picks out some of

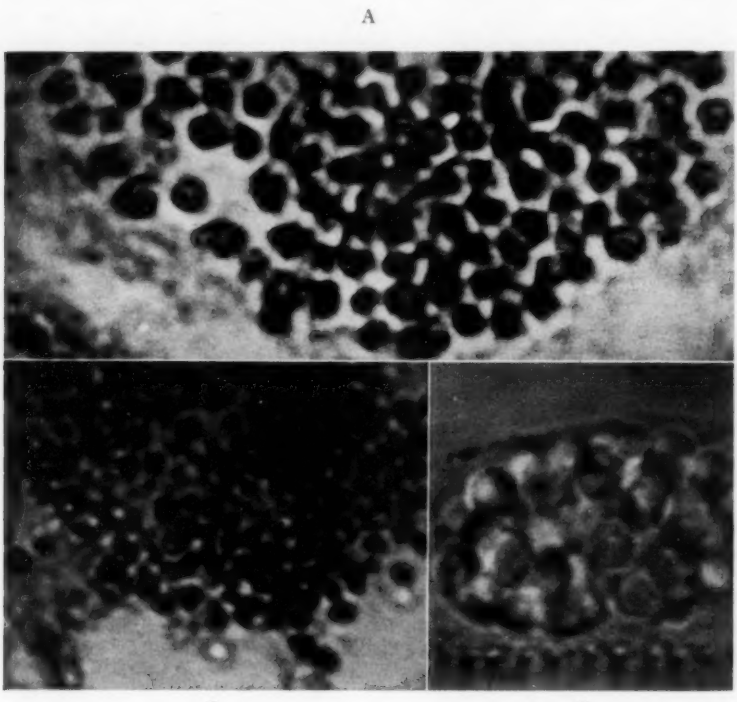


FIG. 4. Nuclei of wilted army worm and gipsy moth caterpillars.

Polyhedral bodies in three dimensions with numerous indications of facets in black or white and sharp dihedral edges. *A*, polyhedra in half the nucleus of a fat cell of an infected army worm × 1500: enlarged from the photographic plate reproduced in Glaser and Chapman (1916) as Fig. 3, Plate II. *B*, half of smaller nucleus of same × 1416. *C*, polyhedra in nucleus about tracheal tube of gipsy moth caterpillar × 1875: enlarged from the original reproduced as Fig. 2, Plate II, in Glaser (1915). As now printed, the above enlargements are reduced one-half.

the trihedral angles. These appear as minute points from which may radiate three slightly luminous edges. Photographs in which the objects are vaguely outlined by means of illuminated facets, trihedral angles, and edges are shown in Fig. 6. The hexagonal distribution of the trihedral angles and the square facets, each outlined by four luminous points, are especially noteworthy.

FIG. 5. Hexagonal and square orientations of polyhedral bodies.

The large hexagon is viewed by transmitted light. It is equilateral and the facial angles are 120°. The square orientations demonstrate equal sides with 90° angles. In many instances the upper levels of the adjacent lateral hexagons are partially lighted. The octagonal marginal outlines are visible whenever the optical sections cut hexagonal facets parallel to their diameters and square faces parallel to their diagonals.

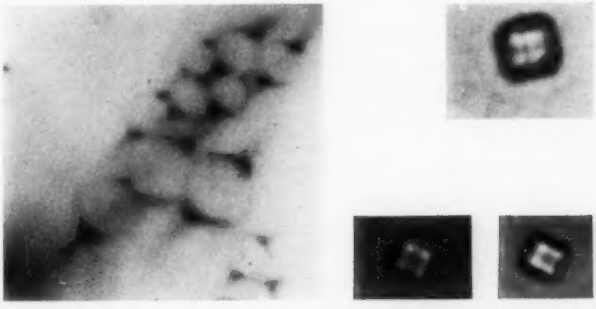


FIG. 6. Polyhedral bodies of the silk worm at various magnifications showing facets, trihedral and dihedral angles, under lateral illumination.

IV

For our immediate purposes it is unnecessary to know the chemical composition of the intra-nuclear hexoctahedra of insects, or whether these objects are crystals (*cf.* Glaser and Chapman, 1916). It is important that bodies with this shape stack without waste space; that slightly rhombic forms can be treated as if they were orthic (Kelvin,

op. cit.) ; and that the properties of cellular emulsions and the pressures to which they may be subjected are such that hexoctahedra of all possible orders of magnitude and all degrees of perfection may be postulated. Many of these may be only rough temporary approximations; others may be expected whenever the emulsions of which they were parts are properly compressed and undergo fixation. Conceivably even true crystals may appear under conditions that would impose the same form on non-crystalline materials.

Systems of this sort may not be as regular throughout as the polyhedra within a given insect nucleus; nevertheless even yolk contains regions of essentially orthic units constant in size and presumably constant in weight. Whether aberrancies cancel out in sufficiently voluminous masses or whether the polyhedra or their precursors in a given system may deviate from the hexoctahedral ideal by constant fractions, the fact remains that irregularities prove relatively unimportant.

V

Stacked in n layers about a central unit, the total number of individuals, Sn, in an isogonic aggregate of orthic hexoctahedra is $4n^3 + 6n^2 + 4n + 1$ (Marvin, 1936). This statement is identical with

$$Sn + n - 0.5 = \frac{(2n+1)^3}{2}.$$

Essentially, therefore,

$$\log\ Sn = 3\ \log\ (2n+1) - 0.3010.$$

With this transformation hexoctahedral aggregation assumes a formal identity with organic growth,

$$\log\ w = k\ \log\ (2t+1) + C,$$

in which weight is equated against time (Glaser, 1938, in press). Hence, if at some level within the cell, organic growth is controlled by hexoctahedral aggregation, layer number in such aggregates must be proportional to time.

VI

This proportion suggests an absolute standard for heterogonic correlations since the heterogony constant k_h in $y = bx^{k_h}$ is the ratio between the growth constants for x and y (Huxley, 1932; Glaser, 1938). Without identifying x or y with the units that control their magnitudes, it is possible to attribute the serial changes in x or y to a summation with

properties indistinguishable from those of the hexoctahedral aggregation series. Accordingly, this series must be able to replace either organic correlative in the heterogonic equation.

When the ages of x or y are unknown, proper adjustment between the hexoctahedral series and the organic correlates may be possible by trial and error; when the organic weights are dated, the proportionality between layer number and time is decisive.

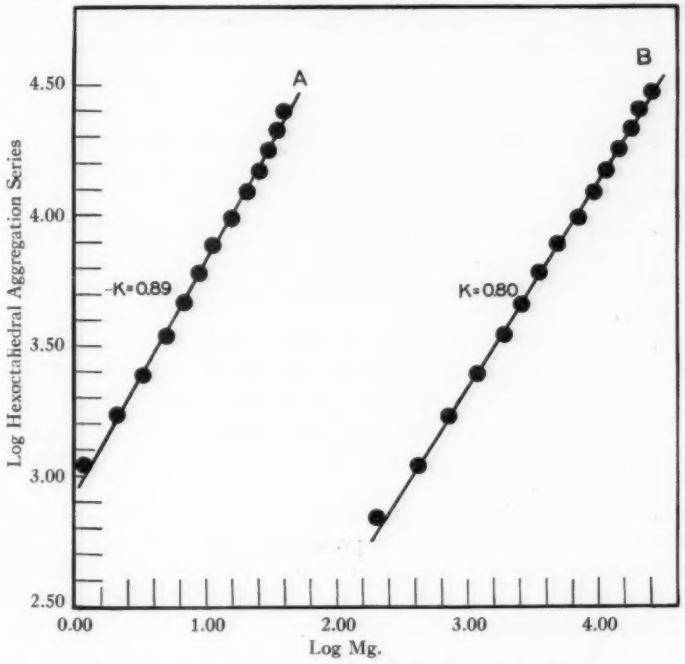


FIG. 7. Heterogonic plot. Two organic weight series against the ideal hexoctahedral aggregation series. *A*, the quantitatively organized chlorides in the chick embryo, based on Table I, Murray (1926, p. 794). *B*, fresh weight of chick embryos based on the original analyses of Murray (1926; Table III, p. 410).

Substitutions of the dated variety are illustrated in Fig. 7. Curve *A* shows the relation between the quantitatively organized chlorides of the chick embryo and the Marvin series; *B*, the relation between this series and the weight of the entire chick. In both instances the linear relationship implicit in $y = bx^k{}_h$ is fairly realized. Moreover, since the growth constant for the chick is 3.76 (Glaser, *op. cit.*, in press) the hexoctahedral heterogonic constant should be $\frac{3.00}{3.76} = 0.797$. The inde-

pendent graphic determination yields 0.80. For the chlorides with a growth constant of 3.53 (Glaser, *op. cit.*) the constant for curve *A* should be $\frac{3.00}{3.53} = 0.849$. Independent graphic determination indicates 0.89.

Many other quantitative relationships of the types here illustrated can be predicted and, where the data permit, verified. For this reason we may consider the hexoctahedral aggregation series as an instrument which enables us to penetrate to analytical levels not attainable without reference to the geometrical foundations of organic cumulative growth.

Summary

1. Certain starch grains and the polyhedral bodies of insects are hexoctahedra of the type described by Lewis for tissue cells and coagulated yolk, and by Kelvin in systems of soap bubbles.

2. Compression of units visible in cellular emulsions results in aggregates of hexoctahedra. Under the same conditions the same form is postulated as either imminent or actual, at levels below the limits of visibility.

3. If this extension applies to materials that control the dimensions of organisms, Marvin's hexoctahedral aggregation series should be capable of replacing either organic correlative in the heterogonic equation $y = bx^{k}_{A}$. Evidence is afforded by heterogonic curves in which the Marvin series is plotted against the chick embryo and the chlorides of the chick embryo.

4. The success of this test depends on two facts: (*a*) the heterogonic constant is the ratio between two growth constants; and (*b*) when isogonic aggregates of hexoctahedra are produced by organic growth, the number of layers in which the resulting units stack about a central unit, is proportional to time.

REFERENCES

Glaser, R. W., 1915. Wilt of gipsy moth caterpillars. *Jour. Agr. Res.*, **4**: 101.

Glaser, O., 1938. Growth, time, and form. *Biol. Rev.*, **13**: 1.

Glaser, R. W., and J. W. Chapman, 1913. The wilt disease of gipsy moth caterpillars. *Jour. Econ. Entomol.*, **6**: 479.

Glaser, R. W., and J. W. Chapman, 1916. The nature of the polyhedral bodies found in insects. *Biol. Bull.*, **30**: 367.

Huxley, J. S., 1932. Problems of Relative Growth. Methuen and Co., London.

Kelvin (Sir W. Thompson), 1887. On the division of space with minimum partitional area. *Phil. Mag., Series 5*, **24**: 503.

Lewis, F. T., 1923. The typical shape of polyhedral cells in vegetable parenchyma and the restoration of that shape following cell division. *Proc. Am. Acad. Arts and Sci.*, **58**: 537.

LEWIS, F. T., 1926. A further study of the polyhedral shapes of cells. *Proc. Am. Acad. Arts and Sci.*, **61**: 1.

LEWIS, F. T., 1933. The significance of cells as revealed by their polyhedral shapes, with special reference to precartilage, and a surmise concerning nerve cells and neuroglia. *Proc. Am. Acad. Arts and Sci.*, **68**: 251.

MARVIN, J. W., 1936. The aggregation of orthic tetrakaidecahedra. *Science*, **83**: 188.

MATZKE, E. B., 1927. An analysis of the orthic tetrakaidecahedron. *Bull. Torrey Bot. Club*, **54**: 341.

MATZKE, E. B., 1931. Modelling the orthic tetrakaidecahedron. *Torreya*, **31**: 129.

MURRAY, H. A., 1926. Physiological Ontogeny, A. Chicken embryos. VII. The concentration of the organic constituents and the calorific value as functions of age. *Jour. Gen. Physiol.*, **9**: 405.

MURRAY, H. A., JR., 1926. Physiological Ontogeny, A. Chicken embryos. XI. The pH, chloride, carbonic acid, and protein concentrations in the tissues as functions of age. *Jour. Gen. Physiol.*, **9**: 789.

POUCHET, F. A., 1847. Théorie positive de l'ovulation spontanée. Paris.

REICHERT, E. T., 1913. The Differentiation and Specificity of Starches in Relation to Genera and Species. Carnegie Institution of Washington, Washington, D. C. Publication 173.

STUDIES ON PRODUCTIVITY AND FERTILITY OF DROSOPHILA MUTANTS

GAIL L. CARVER

(From Mercer University, Macon, Ga.)

Introduction

From his tests of the mutant "Truncate" in various crosses, Hyde (*Jour. Exper. Zool.*, **17**: 173) concluded that the degree of fertility of each gamete was directly dependent upon the genes carried by that gamete, irrespective of the zygote producing it. On the other hand, the crosses carried out by Morgan (*Zeitschr. Abst. Vererb.*, **7**: 323) with the sex-linked recessive "rudimentary" had led to the hypothesis that "prematuration" effects of the zygote upon the developing germ cells explained the peculiar partial sterility of the rudimentary females. Accordingly, a study of the effects of gametic versus zygotic constitution on the productivity and fertility of various mutants was undertaken. These tests were started at Columbia University but were mainly made at Woods Hole during five summers there. I wish to express my sincere thanks to Professor Morgan and Dr. Bridges for their advice and help in this analysis.

Productivity of Vestigial

The first mutant investigated was the second chromosome recessive "vestigial wings" which had given evidence of low productivity not complicated by obvious sterility of any sort. In order to avoid high or aberrant mortality through overcrowding or underfeeding of the growing larvae, the individual females and their mates were transferred every few days to fresh culture bottles of large size liberally supplied with food. The total productivity of each female was obtained by continuing the transfer as long as the female lived. Table I gives summaries of the productivities of the wild stock control and of the crosses involving vestigial.

The most striking relation evident in the result is that the productivity is dependent primarily on the zygotic type of the mother. The cultures from vestigial mothers averaged 344 (51 vg ♀ with 17,538 offspring) not much more than half the average of 581 from non-vestigial mothers (72 non-vg ♀ with 41,897 offspring). But the average length of life of the vestigial mothers was 28 days while the average life of

the non-vestigial mothers was 51 days. That the difference in productivity was mainly due to this difference in life-span was shown by the fact that the daily productivity averages 12.3 for the vestigial and 11.4 for the non-vestigial mothers.

In the results of Table I, a second striking relation is shown, viz.: that productivity rises with outcrossing to diverse strains. Thus, while the productivity of the females of the inbred Falmouth strains averaged 534, the productivity rose to 612 when outcrossed to another wild strain and to 611 when outcrossed to vestigial males. Similarly, the productivity of vestigial females of the inbred cross was only 283, but females of this same stock gave a productivity of 426 when outcrossed to Falmouth males and our intermediate productivity of 373 when outcrossed to F_1 heterozygotes. That the improvement with outcrossing

TABLE I

Crosses testing productivity of vestigial and wild

♀ ♂	Pairs	+ ♀	+ ♂	Vg ♀	Vg ♂	Total	Average Production
Fal. × Fal.	37	10,053	9.677	—	—	19,730	534
Fal. × W. H.	9	2,828	2,663	—	—	5,491	612
Fal. × vg	10	3,120	2,985	—	—	6,105	611
+/vg × vg	16	2,742	2,611	2,678	2,540	10,571	661
vg × vg	21	—	—	3,004	2,937	5,941	283
vg × Fal.	10	2,153	2,106	—	—	4,259	426
vg × +/vg	11	1,022	1,033	1,057	988	4,100	373
F_2vg × F_2vg	9	—	—	1,645	1,593	3,238	360

is due to the genetic constitution of the hybrids as compared with the parental races seems clear and is analagous to the "heterosis" effect observed in corn strains. Further evidence of genetic influence upon productivity is seen in the fact that vestigials extracted from the F_2 gave an average productivity of 360—higher than the productivity of the vestigial stock. The improvement seen in the extracted vestigials must be due to genes that assort separately from the vestigial gene.

THE FERTILITY OF EGGS FROM THE VESTIGIAL CROSSES

Besides length of life and average daily output of eggs, another factor in productivity is the percentage of eggs that give adults the fertility of the eggs. Hyde had introduced the method of isolating counted eggs and observing how many adults these produced. Similarly, I studied fertility by isolating each female and her mates in a

bottle and collecting her eggs upon a strip of blotting paper thickly spread with fermented banana. These strips were changed twice daily, the eggs counted and transferred to well-fed culture bottles where larvæ developed. By additional food supplied as the larvæ grew, mortality, in the post-egg stage, was minimized and the differences in egg fertility brought out clearly. In Table II are summarized the results of the fertility studies on crosses involving vestigial and the wild stock Falmouth.

TABLE II

Crosses testing fertility of vestigial and wild

♀ × ♂	Eggs	Adults	Fertility
			per cent
Fal. × Fal.	3,505	2,080	59
Fal. × +/vg	1,995	1,456	73
Fal. × vg	2,619	1,868	71
+/vg × Fal.	1,801	1,475	82
+/vg × +/vg	3,240	2,070	64
+/vg × vg	2,066	1,441	70
vg × Fal.	1,617	1,069	66
vg × +/vg	1,973	1,164	59
vg × vg	4,149	1,897	46
F_2vg × F_2vg	640	465	55

The significant feature of the results of Table II is the parallelism shown between the fertilities and the productivities of Table I. Thus the fertility of the Falmouth inbred stock was 59 per cent which was raised to 71 per cent and 73 per cent when these same females were outcrossed to vestigials or to heterozygotes. Similarly, the fertility of the vestigial females was raised from 41 per cent to 66 per cent and 59 per cent when outcrossed to Falmouth and to heterozygous males—the increase in fertility being similar in amount to the increases in productivity and presumably accounting for their occurrence. The fertility of the heterozygous females mated to wild males was markedly better (82) than that of the wild females (59) or vestigial females (66) mated to wild males. Finally, the fertilities of the F_2 extracted vestigials (55) was better than that of the inbred stock (46) and was matched by the increased productivity noted in Table I.

Hence, it may be concluded that the primary genetic difference between the vestigial and wild stock (the vestigial gene) was responsible for the curtailment of productivity of vestigial females to approxi-

mately half that of wild through its determination of a phenotype not able to function for much more than half the normal span of life. The two original stocks differed in secondary gene pairs which (1) heightened the productivity of crosses by improved viability of the hybrids in the egg stages, (2) heightened the productivity of hybrids (heterosis effect) and which (3) were able to redistribute with respect to the vestigial pair and bring about the improved fertility and consequent improved productivity of the F_2 extracted vestigials.

Fertility of the High-producing "Hairy" Strain

Unusually high production had been noted for a few strains, notably for the heterozygotes of hairy. A study of the fertility of hairy (Table III) showed that the outcross of hairy by Falmouth gave an increased fertility of 64 per cent as compared with the 59 per cent of Fal. × Fal. and the 60 per cent of hairy × hairy. The hybrids themselves gave an even higher fertility of 73 per cent corresponding to the unusually high productivity originally observed.

Table III

Fertility of hairy and wild

♀ × ♂	Eggs	Adults	Fertility
			per cent
Fal. × Fal.	3,505	2,080	59
h × Fal.	1,157	743	64
h × h	1,706	1,032	60
+/h × +/h	679	494	73
F_2 × h	4,527	2,747	61
F_2h × h	2,625	1,459	56

The Fertility Relations of Singed and Singed^3

Among the mutant strains at Columbia there were three which showed complete or nearly complete sterility of the females but for which there existed allelomorphic strains similar in phenotypes but with approximately normal fertility of the females.

One such set of allelomorphs was singed and singed^3, sex-linked recessive found by Mohr. In both mutants, the bristles and hairs are tightly curled or crumpled, but singed^3 has normal fertility while singed females lay few eggs all abnormal in shape and filaments.

As shown in Table IV, the abnormal eggs laid by young singed females averaged only about 7 per day as compared with 20 per day

for singed[3] females and 25 per day for their hybrids. None of the eggs of the singed females gave offspring while the fertility of the eggs of singed[3] females was 33 per cent with brothers and 53 per cent in crosses to singed males. Fertility of the hybrids was 38 per cent and 37 per cent when mated to singed and singed[3] males—better than that of either pure race.

TABLE IV

Fertility of singed and singed[3]

♀ × ♂	Eggs	Eggs Daily	Adults	Fertility
				per cent
sn × sn	754	6.6	0	0
sn × sn[3]	1,023	7.8	0	0
sn[3] × sn[3]	3,272	20.4	1,085	33
sn[3] × sn	3,269	19.2	1,718	53
sn/sn[3] × sn	2,903	24.4	1,093	38
sn/sn[3] × sn[3]	3,034	25.9	1,112	37

FERTILITY RELATIONS OF REDUCED AND SCRAGGLY

The second—chromosome-recessive allelomorphs "reduced" (rd) and "scraggly" (rd[s]) both found by Bridges, are characterized by irregular and variable reduction in number and size of the bristles. Scraggly is of normal fertility but females of reduced lay only a few eggs which almost never give offspring though they are normal in appearance.

TABLE V

Fertility of reduced and scraggly

♀ × ♂	Eggs	Adults	Fertility
			per cent
rd × rd	460	0	0
rd × rd[s]	540	4	1
+/rd × rd	385	89	23
+/rd × rd[s]	1,305	563	43
rd[s] × rd[s]	1,299	526	40
rd[s] × rd	688	315	46
rd/rd[s] × rd	3,983	1,162	29
rd/rd[s] × rd[s]	4,560	996	22

As shown in Table V, the eggs from reduced ♀ × reduced ♂ gave no offspring, while reduced ♀ out-crossed to scraggly ♂ gave only 4 offspring. That the poor fertility of reduced is semi-dominant seems

probable from the sub-normal fertility of heterozygous rd/+ ♀♀ × rd ♂♂ (23 per cent) and × rd^s ♂♂ (43 per cent), though, as usual, the outcross gave the higher fertility. Similarly, the semi-dominance of the poor fertility of reduced may account for the fact that the rd/rd^s females gave only 29 per cent and 22 per cent fertilities, lower than the fertility of scraggly females (40 per cent inbred and 46 per cent outcrossed). Within each fertility test involving reduced or scraggly, a high degree of variability appeared, with a tendency for the fertilities of individual pairs to fall into three groups—one below 5 per cent, one centering around 20 per cent and one around 50 per cent.

Fertility of Rudimentary^7 and Rudimentary^{14}

The original mutation rudimentary had been lost, but several new allelomorphs had been found by Bridges. Among them, rudimentary^{14} had the very short wings and female sterility characteristics of the rudimentary, while rudimentary^7 had wings only slightly shorter than normal and fertility not obviously reduced. The eggs of both strains are normal in appearance.

Table VI

Fertility of Rudimentary^7 and Rudimentary^{14}

♀ × ♂	Eggs	Eggs Daily	Adults	Fertility
				per cent
$r^{14} \times r^{14}$	2,390	13	0	0
$r^{14} \times r^{7}$	2,485	14	3	0+
$r^{7} \times r^{7}$	3,016	18	1,449	48
$r^{7} \times r^{14}$	2,194	18	1,263	58
$r^{7}/r^{14} \times r^{14}$	6,562	40	2,883	44
$r^{7}/r^{14} \times r^{7}$	3,413	35	1,886	55

As seen in Table VI, rudimentary^{14} ♀♀ laid an average of 13.5 eggs per day, though none gave offspring in the mating to rudimentary^{14} ♂♂ and almost none in the outcrosses to rudimentary^7 ♂♂. Rudimentary^7 ♀♀ gave more eggs per day (18) and these had the nearly normal fertility of 48 per cent upon inbreeding and 58 per cent upon outcrossing to rudimentary^{14}. The r^7/r^{14} hybrids gave egg outputs of 39 ± per day, higher than either parent. The fertility of the hybrids, 44 per cent, and 55 per cent, show only a little lowering from the rudimentary^7 standard through the substitution of the infertile rudimentary^{14} gene whose effects are nearly recessive.

Correlated Variability of Phenotype and Fertility

The phenotype variability of vestigial, hairy, singed and singed[3] was very slight under the stable culture conditions of the experiments and correspondingly the productivity and fertility were highly uniform for the different individual mating within each type of cross.

The reduced and scraggly phenotypes showed considerable variability both in the bristly size and number and in the fertilities. Even more variable in phenotypic expression are rudimentary[7] and rudimentary[14], and the greatest variability in wing size and venation reduction was shown in the r^7/r^{14} hybrids. Corresponding fairly close to the degree of phenotypic departure was the reduction in productivity of the rudimentary[7] and r^7/r^{14} hybrids. Nine hybrids, selected for their extreme shortness of wings, gave 626 eggs from which only one offspring emerged—thereby resembling their rudimentary[14] parent in both phenotypes and productivity.

Discussion

The results of the experiments are all compatible with the view that fertility of gametes is independent of their genic content but is determined by the phenotype of the zygote producing them. Even in normal races, a surprisingly high proportion of eggs, about 40 per cent, may fail to produce adults. It would seem that the stages of fertilization and early embryonic development must be highly unstable and precarious. That the genetic composition of the zygote influences strongly the early process of development is evident from the practically universal improvement in fertility and productivity brought about by outcrossing. Other evidences of the rôle of inheritance in the fertility—increased fertility of the hybrids and changed fertility of extracted types—point to a situation for *Drosophila* analagous to that in maize, where inbred strains differ by large numbers of genes with slight individual but larger cumulative effects.

THE pH OF SEA WATER AS MEASURED WITH THE GLASS ELECTRODE

ERIC G. BALL AND C. CHESTER STOCK

(From the Marine Biological Laboratory, Woods Hole, Mass., and the Department of Physiological Chemistry, the Johns Hopkins University School of Medicine, Baltimore, Maryland)

Most methods available for the determination of pH must be subjected to critical study when applied to sea water. The reason lies in the fact that sea water is a relatively weakly buffered medium with a high salt content. For instance, the application of the colorimetric method to sea water has required the evaluation of rather large correction factors for the so-called salt error. In the case of the quinhydrone electrode, the determinations of sea water are not only subject to a salt error but accuracy is also impaired by the inherent instability of quinone at the pH values encountered. The method of choice would appear to be the glass electrode since it is highly suitable to unbuffered solutions. It is, however, well known that the glass electrode is susceptible to salt effects particularly in the alkaline range. It is the purpose of this paper to show that the salt content of sea water does not interfere with the application of the glass electrode to the determination of its pH and that such determinations may be made with a fair degree of accuracy.

Procedure

The method of reference in all pH determinations is the hydrogen electrode. It would therefore appear that the simplest procedure for checking the accuracy of the glass electrode would be parallel determinations on sea water by both procedures. Such a study is unfortunately open to experimental difficulty by reason of the fact that the CO_2 tension of sea water and thereby its pH is altered while developing the atmosphere of hydrogen necessary to the use of the hydrogen electrode. Though this experimental difficulty is surmountable by the use of special apparatus of the type described by McClendon (1917), it was felt that a simpler though valid test of the effect of sea salt on the glass electrode might be furnished by parallel determinations by both methods on sea water deaerated with hydrogen. Even though the pH of sea water so treated is altered toward more alkaline values a sample uniformly treated should serve for an adequate comparison of the two

methods. It was, however, impossible to obtain with the hydrogen electrode consistent values upon sea water so treated. The poor buffering capacity of such samples may be responsible. An indirect attack upon the problem not unlike that used by Sørensen and Palitzsch (1910) in their standardization of the colorimetric method for use with sea water was therefore adopted. This involved the addition of an amount of dry sea salt to various buffer solutions so as to give a concentration of sea salt equal to that found in sea water. The pH values of the buffer solutions were determined both before and after the addition of the sea salt using both the hydrogen and the glass electrode. The sea salt was obtained by evaporation of sea water on a water bath, any organic matter that precipitated out during the heating was filtered off. The salt so obtained was dried at 130° C. A liter of sea water yielded 33.1 grams of dry salt.

The hydrogen electrode employed in the determinations was the Clark type shaking vessel. A saturated KCl-calomel half-cell was used which was standardized against a standard acetate buffer, which in turn had been checked against a 0.1 molal KCl-calomel half-cell. The tentative standard of potential proposed by Clark (1928) was used. The hydrogen used was deoxygenated by passing it over heated (850° C.) platinized asbestos. The potentiometer circuit was the orthodox one, a galvanometer being used as a null point instrument.

Two types of glass electrodes were used. What will be designated hereafter as Type I was a commercial apparatus known as the Beckman pH meter. In this glass electrode the glass membrane separates a quinhydrone electrode from the sample to be measured which in turn makes a liquid junction with a saturated KCl-calomel half-cell. The amplifier and potentiometer circuit were not available from the manufacturer. The outfit was used without any modifications. In the other apparatus (Type II) we employed a Leeds and Northrop, No. 7673 vacuum tube amplifier in conjunction with the potentiometer and galvanometer of the hydrogen electrode circuit. The glass electrode, which was blown from Corning 015 glass, separated a Ag-AgCl half-cell from the sample and a saturated KCl calomel half-cell. The junction between the sample and saturated KCl was made at a stopcock of the type employed by Stadie, O'Brien, and Laug (1931).

All determinations were carried out in a room which was automatically kept at a relative humidity of 50–60 per cent. Rigid temperature control was not maintained though the variation during a series of readings was never more than 0.1° C. The control of humidity was found to be an important item in the reliable use of glass electrodes of the type employed under the climatic conditions of the seashore.

Results

In Table I are summarized the results obtained on buffer solutions with and without the addition of sea salt. Each pH value given for the hydrogen electrode is the average of triplicate determinations while the glass electrode results are the average of duplicates. The hydrogen and glass electrodes agreed within the experimental error in all cases except that of the borate buffer with added sea salt. Here the glass electrode readings tended to fall a little below those of the hydrogen electrode. This may be the result of a salt effect but it should be noted that the pH has shifted toward more alkaline values where the glass electrode

Table I

The pH of buffer solutions as affected by the addition of sea salt.
Temperature, 25° C.

Buffer	Hydrogen Electrode	Glass Electrode No. 1	Glass Electrode No. 2
HCl	1.04	1.02	1.04
HCl + salt*	1.12	1.11	1.12
Acetate	4.60	4.59	4.61
Acetate + salt*	4.58	4.59	4.61
Phosphate	6.99	6.99	6.99
Phosphate + salt*	6.72	6.70	6.70
Borate	9.13	9.13	9.13
Borate + salt*	9.51	9.46	9.47

* Dried sea salt added to a known volume of buffer in amounts equal to that present in the same volume of sea water.

usually begins to deviate below the theoretical. The pH shift produced by the addition of sea salt to the borate and phosphate buffers is partly to be attributed to the precipitation of some of the buffer components by the Ca and Mg salts present in the sea salt. This precipitate was filtered off before making the measurements recorded here. Another contributing factor to this pH change is undoubtedly the change in activity coefficients brought about by the higher ionic strengths of the solutions. This factor and the volume changes that may have occurred on addition of the dry salt to the buffer solutions, are largely responsible for the pH shift in the case of the acetate and HCl buffers since no precipitates occurred in these solutions.

We feel that these results justify the direct use of the glass electrode for the measurement of the pH of sea water. In drawing this conclusion we are mindful of the fact that the precipitation of some Ca and Mg salts in the two most alkaline buffers has somewhat distorted the ion balance from that found in sea water. It is doubtful, however, that the presence or absence of such small amounts of these large divalent ions would modify perceptibly the behavior of the glass electrode when much larger quantities of the smaller univalent sodium ion have no pronounced effect. Dole (1937) has stated that "the cation error is greatest with those cations that most easily penetrate the glass, sodium and lithium being the worst offenders, while large ions, such as potassium, and also divalent ions have only a small influence."

TABLE II

The pH of sea water as measured with the glass electrode. All samples collected September 10, 1936, Woods Hole, Mass.

Sample No.	Source	Water Temperature	pH
		°C.	
1	Great Harbor * Laboratory Dock	20.0	8.13 8.14
2	Tank supply (Room 333)	20.0	8.05 8.05
3	Tank supply (Room 110)	20.0	8.05 8.05
4	Great Harbor * Laboratory Dock	20.0	8.14 8.14
5	Eel pond *	21.5	7.99 7.98

* Surface water.

In Table II are summarized a few determinations on different samples of sea water. These results were obtained with the Type I glass electrode. Of interest is the difference in pH between samples drawn from the tank system supplying the laboratories, the water for which is pumped from the harbor, and samples collected directly from the harbor. This difference is well outside the maximal experimental error ($\pm$ 0.02). Since all samples were collected in the order given, this difference cannot be attributed to a sudden change in electrode behavior because Samples 1 and 4 are in good agreement. Moreover, the agreement obtained on

Samples 2 and 3 collected in different parts of the laboratory building would indicate that local contamination of the system was not responsible. Since the feed pipes are mainly of lead it is possible that the acid swing is due to the removal of carbonate as an insoluble lead salt. This seems unlikely, however, in view of the fact that such lead feed pipes after continued use accumulate a protective coating of such a precipitate which should prevent further interaction. Another possible explanation for the difference may be the fact that though the two sets of samples are taken from the same locality, one is pumped from a depth and the other is surface water. Though the two samples registered the same temperature at the time of collection, the water from the greater depth may have possessed a lower temperature before its passage through the storage and feeder system. If so, a more acid pH would be encountered due to the lag in the readjustment of the carbon dioxide equilibrium as the temperature increased, a process which Irving (1925) has shown to be slow. The greater acidity of the water collected from the eel pond is to be expected in view of the more stagnant condition of this body of water.

The glass electrode has been used by Mitchell and Taylor (1935) to measure the pH of sea water at different dilutions in order to determine the dissociation constant of cresol red in sea water of different salinity. This work was confirmed and extended and, in addition, data upon phenol red were obtained by Mitchell, Buch, and Rakestraw. Since these authors used a glass electrode with a thin film membrane in contrast to the heavier type employed by us, we wish to emphasize the fact that our proof of the validity of the glass electrode values on sea water applies to this type of membrane only. It would be desirable to have a similar study made with the thin film type of membrane.

Summary

The hydrogen electrode and the glass electrode registered approximately the same pH values for various buffer solutions in which dry sea salt was dissolved to give a concentration similar to that found in sea water. A direct comparison of the two methods on sea water was not possible because the behavior of the hydrogen electrode was erratic in this poorly buffered medium. It is concluded that the salt content of sea water does not interfere with the application of the glass electrode to the determination of the pH of sea water and that such determinations may be made with a fair degree of accuracy ($\pm$ 0.03 pH). The pH value of several samples of sea water have been determined and their differences discussed.

BIBLIOGRAPHY

CLARK, W. M., 1928. The Determination of Hydrogen Ions. Williams and Wilkins, Baltimore, 3rd edition.

DOLE, M., 1937. Measuring pH with the Glass Electrode. Maywood, Illinois.

IRVING, L., 1925. *Jour. Biol. Chem.*, **63**: 767.

MCCLENDON, J. F., 1917. *Jour. Biol. Chem.*, **30**: 265

MITCHELL, P. H., AND I. R. TAYLOR, 1935. *Jour. Conseil. intern. exploration mer.*, **10**: 169.

MITCHELL, P. H., K. BUCH, AND N. W. RAKESTRAW, 1936. *Jour. Conseil. intern. exploration mer.*, **11**: 183.

SØRENSEN, S. P. L., AND S. PALITZSCH, 1910. *Biochem. Zeitschr.*, **24**: 387.

STADIE, W. C., H. O'BRIEN, AND E. P. LAUG, 1931. *Jour. Biol. Chem.*, **91**: 243.

THE RELATION OF ENDOCRINE FEEDING TO REGENERATION, GROWTH AND EGG CAPSULE PRODUCTION IN PLANARIA MACULATA

E. D. GOLDSMITH

(From the Department of Biology, Washington Square College, New York University and the College of the City of New York)

An attempt was made to determine whether the time required for head regeneration in *Planaria maculata* following a series of decapitations would be altered by feeding beef endocrines. In view of the results of Wulzen (1923) on *P. maculata* and Castle (1928) on *P. velata* a record of the size of the individuals was kept. A preliminary report (Goldsmith, 1935) summarized the results; a more complete account follows.

Material and Methods

From a stock of *Planaria maculata* originally collected at Woods Hole, Massachusetts and kept in the laboratory for three months, 204 individuals were selected for experimentation. They were divided into two groups of large and small specimens; those of the large size group (Group *L*) measuring 9–12 mm., those of the small size group (Group *S*) measuring 5–8 mm. in length. The animals of each of the groups were distributed into twelve sets—those of Group *L* containing eight individuals each, and those of Group *S* nine individuals each.

The sets were kept in finger-bowls containing 100 cc. of tap water, and were placed on the following diets:

1. Liver, fresh gland.
2. Liver, aqueous extract.
3 and 4. Anterior pituitary, fresh gland.
5. Anterior pituitary, aqueous extract.
6 and 7. Thyroid, fresh gland.
8. Thyroid, aqueous extract.
9 and 10. Anterior pituitary + thyroid, fresh gland.
11. Anterior pituitary + thyroid, aqueous extract.

The animals in set 12 were not given any food and set 12 is hereafter called the " starved " set.

Fresh beef liver, pituitary and thyroid glands were obtained weekly

at the slaughter house. The first feeding was made within two hours of the obtainment of the material. It was then frozen, so kept for a week during which time it was given to the planarians, and was then replaced by fresh material.

Small pieces of liver and small pieces of thyroid and anterior pituitary, which had been minced, were placed in the dishes and left there for several hours. The food was then removed, the dishes cleaned and fresh tap water added. Feedings were on alternate days and in some experiments daily. In the anterior pituitary + thyroid set the anterior pituitary and thyroid were given on alternate feeding days. The frequent feedings ensured an adequate food supply for the experimental animals.

Another method of treatment was the addition of filtered aqueous tissue extracts to the tap water. Not only were the animals of the aqueous extract sets exposed to the extract but they also ingested some of it as was indicated by the movements of their pharynges.

The thyroid material was tested throughout by treating frog tadpoles with it. Thyroid glands frozen two weeks retained their potency in that tadpoles feeding on this tissue or exposed to aqueous extracts of it of much lower concentration than those used on the planarians showed greatly accelerated metamorphosis.

Using a binocular dissecting microscope the length and width of the animals were measured as they moved on a glass cover-slip mounted over a piece of millimeter cross-section paper. By regulation of the light source the animals could be made to move along the rulings.

Regeneration

Stevens (1901) and others have reported that the eyes in planarians are regenerated more rapidly at an anterior than at a posterior level. It is also known that eyes regenerate more rapidly in a form such as *P. maculata* possessing high regenerative potentialities than in *Procotyla fluviatilis* possessing, in general, low regenerative potentialities.

In the work which follows the time for eye appearance after decapitation was used as an index of the regeneration rate under the influence of different diets. Following each operation the ventral and dorsal aspects of the animals were carefully examined for the presence of eye pigment.

The anterior portions of all animals were removed five times. The specific data of each decapitation are given below.

Group I

Decapitation No. 1.—Thirty-six days after their first feeding the animals were measured. Two days later four animals from each dish were decapitated by a transverse cut directly posterior to the auricles (Fig. 1, *a*) and placed in an additional set of finger-bowls.

The operated animals comprise Group I. Those which were not operated upon were left in their original aquaria and comprise Group II.

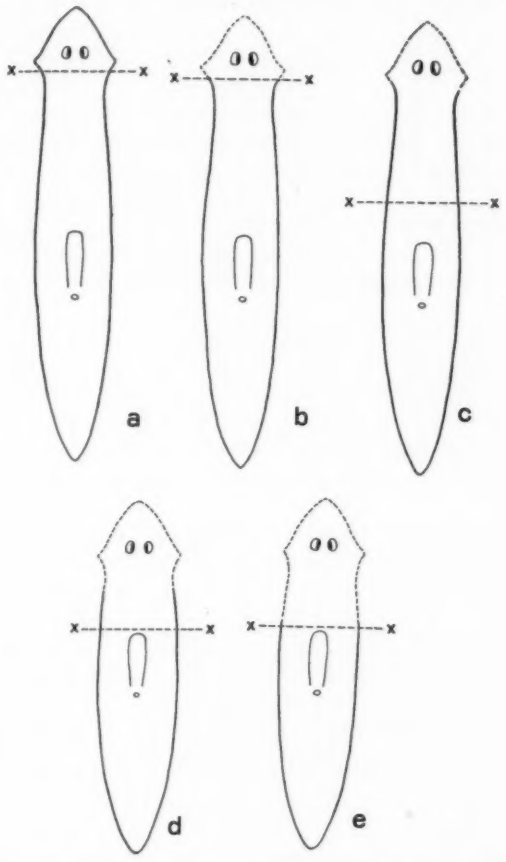


FIG. 1. Decapitations in Group 1. Broken lines indicate tissue regenerated after the previous decapitation. *x-x* indicates level of cut. All figures diagrammatic. *a*, decapitation No. 1; *b*, decapitation No. 2; *c*, decapitation No. 3; *d*, decapitation No. 4; *e*, decapitation No. 5.

Decapitation No. 2.—Fourteen days after the first decapitation the animals were fed. These feedings were continued during a twenty-four-day period, and the regenerated heads were removed as in decapitation No. 1 (Fig. 1, *b*). Food was placed in the dishes and left there for several hours each day throughout the regeneration period.

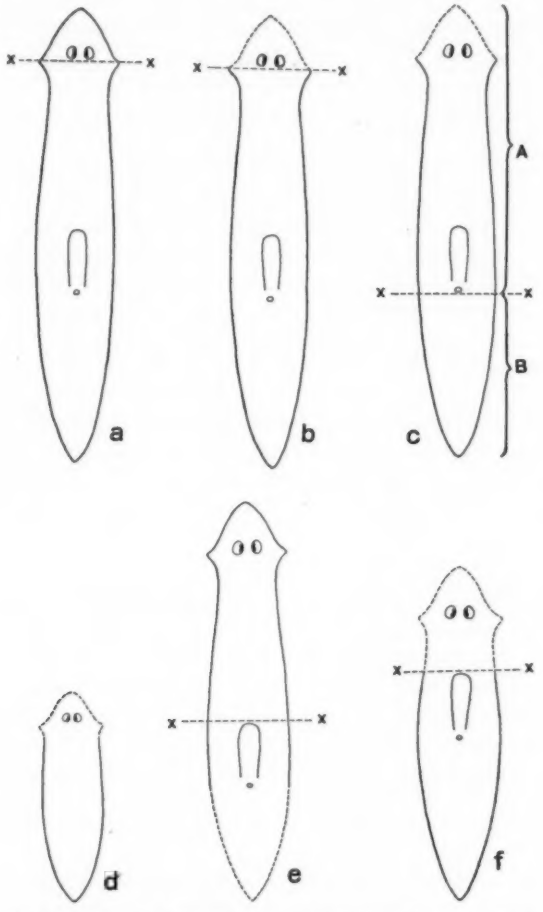


FIG. 2. Decapitations in Group 2. *a*, decapitation No. 1; *b*, decapitation No. 2; *c*, decapitation No. 3 producing *A* and *B* pieces; *d*, portion *B* resulting from decapitation No. 3; *e*, decapitation No. 4 directly anterior to pharynx of portion *A* resulting from decapitation No. 3; *f*, decapitation No. 5.

Decapitation No. 3.—Twenty-one days after the second decapitation the anterior ends were removed by a transverse cut approximately 1 mm. anterior to the pharynx (Fig. 1, *c*). As above, food was placed in the dishes throughout the regeneration period.

Decapitation No. 4.—Twenty-five days after the third decapitation the entire prepharyngeal region was removed (Fig. 1, *d*).

Decapitation No. 5.—The animals were starved for nine days after the fourth decapitation and then all were placed on a liver diet for thirty-five days. Two days after the last feeding the entire prepharyngeal region was removed (Fig. 1, *e*).

Group II

Decapitation No. 1.—Sixty-three days after their first feeding those animals which had not been decapitated previously (Group II) were measured and on the following day were decapitated by a transverse cut directly posterior to the eyes (Fig. 2, *a*).

Decapitation No. 2.—Three days after the first decapitation feeding was recommenced and continued for fifteen days. On the following day the heads were again removed by a cut similar to the first (Fig. 2, *b*).

Decapitation No. 3.—Feeding was begun the day after the second decapitation and continued for nineteen days. The following day a transverse cut was made directly posterior to the pharyngeal pore (Fig. 2, *c*). The posterior portions of the worms (*B*, Fig. 2, *c*) were placed in additional dishes and were observed for eye and head regeneration (Fig. 2, *d*). The anterior portions (*A*, Fig. 2, *c*) possessing the pharynges were fed.

Decapitation No. 4.—Twenty-three days after the third decapitation a transverse cut was made directly anterior to the pharynx (Fig. 2, *e*) in the anterior pieces arising as a result of the previous decapitation (*A*, Fig. 2, *c*).

Decapitation No. 5.—During the eight days following the previous decapitation the animals were not fed. Then they were fed liver on alternate days during a thirty-six-day period. Two days after the last feeding the entire prepharyngeal region was removed by a transverse cut directly anterior to the pharynx (Fig. 2, *f*).

The regeneration rate following the first four operations appeared not to be influenced by the type of food fed if the quantity were suf-

ficient. Following each decapitation the rate of regeneration of a number of the animals varied slightly. The variation in rate within a set of worms on the same diet was as great or greater than the variation among the individuals on different diets (exception noted below).

At the time of the fourth decapitation, 124 days after the first treatment, the animals were in great part composed of tissues built from materials supplied by the particular food to which they were restricted. Notwithstanding this, the results did not differ from those of the previous decapitations.

The exception mentioned above is that of the Liver *L* (gland) set in Group I. Following each of the four operations several worms in this group regenerated more slowly than did any in the other gland sets. In order to ascertain whether this difference in rate was to be attributed to the difference in diet or to an innate tendency of these particular individuals, all of the experimental animals were starved for eight days and then fed only liver for thirty-six days. The anterior ends were then removed (fifth decapitation). Several planarians in the original liver-fed set in Group I, *L*, still regenerated more slowly than the others. Since these animals still lagged behind the others when all individuals in all sets were on the same liver diet, and since the animals in the Liver *L* and Liver *S* (gland) sets in Groups II and those in the Liver *S* (gland) set in Group I did not lag following any of the decapitations, it would appear that the retarded rate was not to be regarded as an indication that thyroid or anterior pituitary glands accelerated or that liver retarded the rate of regeneration. Rather, it would seem that the slowly regenerating animals did so because of innate individual differences.

In Group II, *S*, the animals starved for sixty-four days regenerated more slowly than any of the others. At a temperature of 21° C. eyes made their first appearance as listed:

Solid foods, 140 hours
Aqueous extracts, 160 hours
Starved, 190 hours

In Group II, *L*, the animals starved for sixty-four days regenerated at about the same rate as the others, 142 hours. This may have been due to the larger animals having more of a food reserve.

In Group I, *S*, following the first decapitation thirty-six days after treatment was begun the rate was the same in all groups. Following the second decapitation, at which time the animals had been treated for seventy-five days, the individuals given aqueous extract lagged about twenty hours behind the animals maintained on the fresh gland. The starved forms lagged slightly behind the " extract " forms.

The starved animals and the extract-treated ones which also showed starvation symptoms were fixed and preserved. Those in Group I (*L* and S) were fixed after regeneration had been observed after their second decapitation and those in Group II (*L* and *S*) after their first decapitation.

Retardation after starvation is well known and has been described in planarians by Morgan and in *Triton cristatus* by Morgulis (Morgulis, 1923).

Growth

Measurements made as described in the earlier part of this paper are recorded in Table I.

Table I

The effects of feeding liver and thyroid glands and of starvation upon the growth of Planaria maculata

Food	A Beginning 2/19		B 36 days after A 3/27		C * 27 days after B 4/23	
	Average length	Average width	Average length	Average width	Average length	Average width
	mm.	mm.	mm.	mm.	mm.	mm.
Liver Gland *S*	7.7	1.1(9) †	10.4	1.2(9)		
			10.2	1.2(5)	12.1	1.8(5)
Liver Gland *L*	10.3	1.4(8)	13.3	1.6(8)		
			13.5	1.6(4)	15.5	1.9(4)
Thyroid Gland *S*	8.0	1.0(17)	8.9	1.1(17)		
			8.8	1.1(9)	9.8	1.4(9)
Thyroid Gland *L*	10.0	1.4(16)	11.6	1.3(16)		
			11.4	1.3(8)	11.6	1.5(8)
Starve *S*	7.7	1.1(9)	6.1	0.6(9)		
			5.7	0.6(5)	3.8	0.4(5)
Starve *L*	10.7	1.6(8)	8.9	0.9(7)		
			8.6	0.9(3)	6.6	0.7(3)

* A number of animals in each set were decapitated on 3/29. The unoperated animals were again measured on 4/23.

† Figures in parenthesis indicate number of animals in set.

It is clear that the liver-fed animals show the greatest increase, and that thyroid gland, while not causing as much of an increase as liver, certainly does not cause the animals to become smaller than starved individuals as is the case in *P. velata* (Castle, 1928).

A number of worms newly emerged from their capsules were also found to increase in length and width when given only fresh thyroid gland for periods ranging from 15–65 days.

Further evidence that *P. maculata* does not respond to thyroid gland treatment as does *P. velata* is presented by those animals which were on an exclusive thyroid diet for 124 days. During this period their anterior ends had been removed four times with typical regeneration resulting. The animals were in excellent condition, and almost indistinguishable from the liver-fed worms. The only noticeable difference was that the latter were slightly thicker dorso-ventrally.

These results are in agreement with the data presented by Wulzen (Table II, p. 178, 1923).

Egg Capsule Production

During the latter part of March, egg capsules appeared in some of the dishes. Records of the numbers produced are presented in Table II.

Table II

Number of egg capsules produced by Planaria maculata on indicated diets

Number of animals	Food	Number of capsules to 4/22*	Number of capsules to 5/12
9	Liver gland	68	87
18	Anterior pituitary gland	36	40
17	Anterior pituitary-thyroid gland	55	62
18	Thyroid gland	18	20
4	Liver aqueous extract *L*	1	1
5	Liver aqueous extract *S*	0	0
9	Anterior pituitary aqueous extract	0	0
9	Anterior pituitary-thyroid aqueous extract	0	0
9	Thyroid aqueous extract	0	0
9	Starve	0	0

* The animals were decapitated on 4/23. They continued to produce capsules to 5/12.

Comparison of the extremes of the series, the liver-fed with the starved and the extract-treated forms, leads one to believe that low capsule production may be due to a food deficiency. Of the starved and extract-treated forms which were becoming smaller, only Liver Aqueous Extract *L* was productive—a single capsule was produced. The animals in this set were slightly larger than the starved and other extract-treated animals, and smaller than any of those which were fed fresh glands.

The thyroid-fed animals, which were least productive of the gland-

fed animals, were in good condition and increasing in size but were smaller than those on the liver diet. Although the planarians fed anterior pituitary and anterior pituitary-thyroid glands approximated the liver-fed ones in all dimensions, they produced a smaller number of egg capsules than the liver-fed individuals.

It is realized that the evidence is too scanty to permit a definite conclusion. It is interesting to note that Greenberg and Schmidt (1936) described an ether-soluble factor in liver which acts as a growth-promoting agent for *Planaria maculata*. Smith and Seegers (1934) found a principle in liver which acts as a growth-promoting agent and which is, in some way, concerned with the typical functioning of the reproductive mechanism in the albino rat.

The writer is indebted to Professor Robert Chambers for his criticism of the manuscript and to Professor F. Gudernatsch for his counsel throughout the course of this work.

Summary

1. Individuals of *Planaria maculata* were fed on abundant, exclusive diets of beef liver, anterior lobe of the pituitary and thyroid glands. Others were given aqueous extracts of the glands or were completely starved.

2. No significant differences were noted in the head regeneration time of the gland-fed animals following each of five amputations of the anterior region.

3. Decapitated animals which were starved and those which were kept on the aqueous extract diet and which showed starvation symptoms regenerated more slowly than those which were fed the fresh glands. There appeared to be a correlation between the rate of regeneration and the initial size of the animals.

4. Thyroid-fed individuals increased in size but to a lesser extent than the liver and pituitary-fed forms. All were in excellent condition following four decapitations. Individuals newly emerged from capsules also increased in size when fed thyroid gland exclusively.

5. Liver-fed animals produced a greater number of egg capsules than any of the others. The starved and extract-treated forms, with the exception of a single capsule by the Liver *L* Aqueous Extract set, produced no capsules.

BIBLIOGRAPHY

CASTLE, W. A., 1928. An experimental and histological study of the life-cycle of Planaria velata. *Jour. Exper. Zoöl.*, **51**: 417.

GOLDSMITH, E. D., 1935. Regeneration and growth in Planaria maculata under the influence of endocrine feeding. *Anat. Rec.*, **64** (Supp. No. 1): 78.

GREENBERG, L. D., AND C. L. A. SCHMIDT, 1936. Studies on the properties of a growth-promoting factor for Planaria maculata. *Jour. Exper. Zoöl.*, **73**: 375.

MORGULIS, S., 1923. Fasting and Undernutrition. E. P. Dutton Co., N. Y.

SMITH, H. G., AND W. H. SEEGERS, 1934. The nutritive value of animal tissues in growth, reproduction, and lactation. I. Alcohol extracted beef liver. *Jour. Nutrition*, **7**: 195. II. The presence of a new dietary principle in liver. *Jour. Nutrition*, **7**: 209.

STEVENS, N. M., 1901. Notes on regeneration in Planaria lugubris. *Arch. f. Entw.-mech.*, **13**: 396.

WULZEN, R., 1923. A study in the nutrition of an invertebrate, Planaria maculata. *Univ. Calif. Pub. Physiol.*, **5**: 175.

RESPONSES OF MUSCLES OF THE SQUID TO REPETITIVE STIMULATION OF THE GIANT NERVE FIBERS

C. LADD PROSSER AND JOHN Z. YOUNG[1]

(From the Marine Biological Laboratory, Woods Hole, Mass.)

INTRODUCTION

Investigation of the response of muscles to repetitive stimulation of their nerve fibers has shown that there are great variations among different muscles and different animals in the possibilities of facilitation at the neuromuscular junction. Although the response of an intact muscle fiber, normally activated, is probably always maximal (all-or-nothing), yet in some muscles, for instance those of Crustacea, increase in the frequency of stimulation often increases the tension developed on account of the fact that some muscle fibers are not activated by single, or even by few impulses. On the other hand, in vertebrate striped muscle, unless drugged or fatigued, a single nerve impulse excites all the muscle fibers which it reaches (Lucas, 1909; Adrian and Lucas, 1912).

The range of muscles which have been investigated from this point of view is still small, especially among invertebrates, and we have accordingly investigated the muscular response to repetitive stimulation of the giant axons in the stellar nerves of the squid, *Loligo pealii*. These fibers have been shown to innervate the circular muscle fibers of the mantle, and a single condenser discharge, unless of great intensity or duration, sets up in the axon a single impulse which is capable of activating all of the muscle fibers which it reaches (Young, 1937). The great stellar nerve, containing a single giant axon, was prepared for stimulation in the manner described elsewhere (Young, 1937), and the contractions of a portion of the muscles which it innervates were recorded by attachment to a semi-isometric lever.

Repetitive stimulation was applied by condenser discharges through a thyratron circuit. With the intensity constant and supraliminal the frequency was varied up to approximately seventy stimuli per second. Fatigue set in very rapidly at higher frequencies. Sixteen experiments in which the giant fiber of the great stellar nerve was stimulated yielded consistent results.

[1] Assisted by a Fellowship of the Rockefeller Foundation.

Results

A typical experiment is shown in Fig. 1. Development of tension is registered by a downward deflection in these records. The first stimulation was at 13 stimuli per second and the second at 24 per second. Incomplete relaxation between twitches occurred in both, and the maximum tension at 24 per second was very slightly greater than at 13.

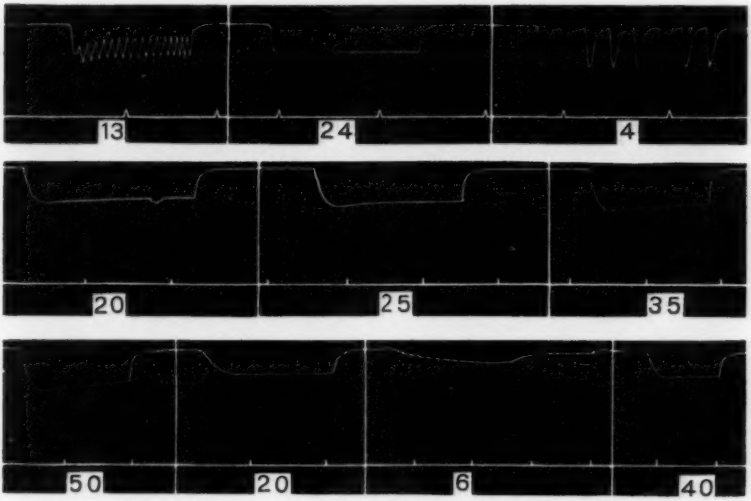


Fig. 1. Record of an experiment in which the giant fiber of the great stellar nerve was stimulated repetitively and the contraction of the mantle muscle was recorded semi-isometrically. Records in order in which they were taken. Numbers indicate frequencies of stimulation.

With stimulation at 4 per second there was complete relaxation between stimuli. In general, complete relaxation between twitches accompanies frequencies of stimulation up to approximately 8 per second. The preparation was then stimulated at 20 and at 25 per second, and showed very slightly increased tension. At 35 stimulations per second fusion of contraction was complete. At 50 per second, as at 35, there was no increase in maximum tension above that at 25 but at 50 the tension declined rapidly, indicating fatigue. The effects of this fatigue were shown when stimulation was returned to 20 per second, where a lower tension was recorded. Thereafter (6 and 40 stimuli per second) the tension varied with the frequency.

Figure 2 shows similar results in three other preparations. In each of these experiments, as in most of the others, there was a slight increase

in tension (5–10 per cent) as the frequency increased during the range of incomplete relaxation, an effect which may be ascribed to the mechanical properties of the muscle.

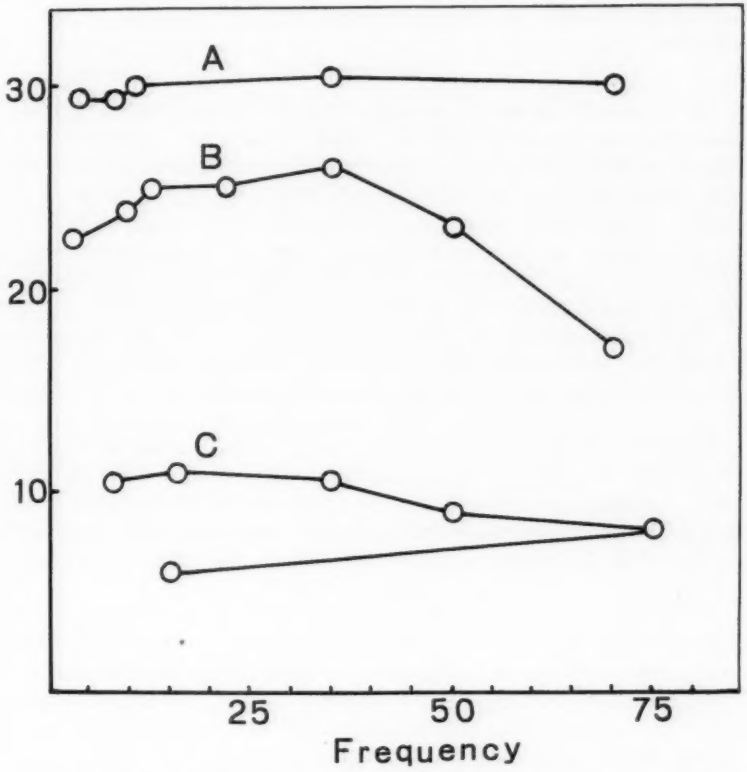


FIG. 2. Plots of maximum tension developed by the mantle muscle against frequency of stimulation of the giant fiber in the great stellar nerve in three preparations. Stimulation proceeded from low to high frequencies. In *C* the lowest point (15 per second) represents response after fatiguing at highest frequency (75 per second).

In Experiment *A*, Fig. 2, the periods of stimulation were brief and there was no increase in tension with increasing frequency of stimulation; this is the general result when no fatigue occurs.

Preparation *B* showed fatigue at 33 stimulations per second, and Preparation *C* at 75 per second. Thereafter the tension fell off and

varied with the frequency of stimulation, the higher frequencies eliciting a greater response than the lower.

The preparation is extremely sensitive to strong excitation and high frequency stimulation during one second causes irreversible fatigue. The failure of the response is parallelled by a growing opacity of the mantle.

Discussion

It is evident that there is normally no facilitation at the junction between the endings of the giant fiber and the muscles. Increased frequency of stimulation produces no increase in tension, indicating that all the muscle fibers are activated by each single impulse which reaches them. Young (1937) observed similar results with increasing intensity of stimulation. In the state of fatigue, however, changes occur, probably at some stage in the contractile mechanism, so that summation occurs and greater tensions are produced at the higher frequencies.

This condition is closely similar to that found in the striped muscles of the frog (see Adrian and Lucas, 1912), but contrasts sharply with that of Crustacea, where a single impulse often elicits no mechanical response (Pantin, 1934, 1936). Thus Katz (1936) found that the tension produced by the flexor muscle of the claw of *Maia* increases nearly ten times when the frequency of stimulation is raised from 50–200 per second. Recent observations by Mr. Grossman in the Physiology Course at the Marine Biological Laboratory indicate that the tension developed by the claw of *Limulus* increases from 25 to 650 grams with a rise of frequency from 1–50 per second.

The absence of such facilitation at the neuromuscular junctions of the giant nerve fiber system of the squid is correlated with the function which the system serves in the animal, namely to produce the contractions by which a jet of water is expelled suddenly from the mantle. Once the contraction has occurred the mantle cavity must enlarge again before further work can be done, and there would be no use for sustained or gradually increasing tensions. The expulsion of each jet of water is a single unitary act, performed in an all-or-nothing manner, and any gradation in speed or distance of propulsion must be obtained by variation in the number of contractions set up.

Summary

With increasing frequency of stimulation of a giant nerve fiber in the squid, *Loligo pealii*, the only increase in the tension developed by the circular muscle fibers of the mantle is a small amount (5 to 10 per cent) over the range of incomplete relaxation. The absence of any increased

response at higher frequencies shows that in the fresh muscle a single nerve impulse is capable of activating every muscle fiber which it reaches.

However, the isolated muscle very readily becomes fatigued when stimulated at high frequency and thereafter greater tension is produced at the higher rates. In the normal animal there would be no use for peripheral facilitation and each contraction of the mantle is produced as an all-or-nothing response.

REFERENCES

ADRIAN, E. D., AND K. LUCAS, 1912. On the summation of propagated disturbances in nerve and muscle. *Jour. Physiol.*, **44**: 68.

KATZ, B., 1936. Neuro-muscular transmission in crabs. *Jour. Physiol.*, **87**: 199.

LUCAS, K., 1909. The "all or none" contraction of the amphibian skeletal muscle fibre. *Jour. Physiol.*, **38**: 113.

PANTIN, C. F. A., 1934. On the excitation of crustacean muscle. I. *Jour. Exper. Biol.*, **11**: 11.

PANTIN, C. F. A., 1936. II. Neuromuscular facilitation. *Jour. Exper. Biol.*, **13**: 111.

PANTIN, C. F. A., 1936. III. Quick and slow responses. *Jour. Exper. Biol.*, **13**: 148.

YOUNG, J. Z., 1937. The functioning of the giant nerve fibres of the squid. In press.

THE OCCURRENCE OF SAPROPHYTIC FUNGI IN MARINE MUDS

F. K. SPARROW, JR.

(From the Woods Hole Oceanographic Institution[1] *and the Botany Department of the University of Michigan*[2]*)*

Introduction

Since it has been recognized for many years that fungi play an important and significant rôle in the disintegration of organic materials in land soils, it is natural to suspect that they might perform a similar function in sea bottoms. Previous papers (Petersen, 1905; Sparrow, 1934, 1936) have shown that in the littoral of certain localities in northern Europe and eastern United States there are true marine fungi which are active in initiating the destruction of living, autophytic marine plants and in certain cases also in aiding in their disintegration. As no systematic study of off-shore localities for the presence of wholly saprophytic fungi in the muds had been reported, the preliminary investigation described in this paper was undertaken during July–August, 1936, at the Woods Hole Oceanographic Institution.

Stations

The following are the locations of stations selected for study and from which the mud cores were obtained. All were in localities marked " sticky " or " sand and mud " on the hydrographic charts.

Station 1. One-half mile N.W. of Weepecket Rock Buoy, Buzzard's Bay. *Depth:* 18.0 meters.

Station 2. Western entrance to Vineyard Sound, 3¼ miles E. of Vineyard Sound Whistling Buoy, 5¼ miles W. by S., ¾ S. on Gay Head Light. *Depth:* 32.7 meters.

Station 3. Gulf of Maine, 45°35′ N., 69°11′ W. (Atlantis Station No. 2640). *Depth:* 163.6 meters.

Station 4. Gulf of Maine, 42°19′ N., 69°20′ W. *Depth:* 220 meters.

Portions of two cores collected by Dr. Henry Stetson from the Gulf of Maine and labelled " Canyon B " (depth 1127.2 meters; Station No. 5, Table I) and " Canyon E " (depth 718.0 meters; Station No. 6, Table

[1] Contribution No. 73.
[2] Contribution No. 630.

I) were obtained from Dr. S. A. Waksman. Since these had previously been partly used for bacteriological purposes and had been stored for some days they were considered questionable sources of data.

Methods and Results

In order to obtain any significant information in the limited time available, the methods outlined below were considered most practical even though they were subject to very definite limitations.

Collection of Cores

The apparatus commonly used in the collection of stratified mud cores for bacteriological purposes was employed. This is a modification of the instrument used by Moore and Neil (1930) in the Clyde Sea area. To eliminate as far as possible contamination during collection, all parts of the sampling apparatus in contact with the sterilized glass tube were thoroughly swabbed with 10 per cent formalin immediately before use. However, trials with swabbed and unswabbed apparatus showed little difference in the number of colonies of fungi obtained. All the usual precautions were employed to keep the cores free from contamination after collection. The lengths of the cores varied but averaged about 12 cm.; their diameter was 18 mm.

Recovery of Fungi

After the tubes containing the cores were brought into the laboratory, the upper cork was flamed, the free water removed, and the supernatant liquid immediately above the mud put in a sterile container for future use. The core was then "blown" under sterile conditions into a sterilized Petri dish and the outside surface and ends removed with a hot scalpel as an additional precaution against contamination during collection.

Two methods were followed in recovering the fungi. The first, practised extensively in the study of fresh-water fungi, involves the use of water cultures. Five jars of sterilized sea water were prepared into each of which was placed a core from Station 2, a site at which cores were readily obtainable. The cores were broken up in the water, the mixture allowed to settle, and 20–30 dead *Calanus finmarchicus* and *Saggita* from Station 2 were added. Three jars were kept at room temperature (23° C.) in the light and two in the dark at about 6° C. Bits of "shiner" (a small marine fish) removed aseptically were also dropped into the cultures. After 10 days no fungi were found on any of this "bait."

All the significant data were obtained by the plate method. The medium used was the following:

Dextrose	10.0 grams
Peptone	2.0 grams
Agar	15.0 grams
Sea water	1000.0 cc.

This gave a reaction of pH 6.1.

In plating out, samples were taken from the supernatant water, the surface, the middle, and the bottom of the core. The water was distributed, undiluted, among five dishes and the medium added. Five samples were taken from each of the three regions of the core. These were mixed with 1 cc. of sterile water and plated out in the usual manner. Each of the mud samples was about the size of a pea and weighed 200–250.0 mg. In many cases pieces of mud were also laid on the solidified medium, but these dishes merely told whether or not fungi were present on or near the face of the sample. Flasks of unsolidified media were also tried but these became too heavily overgrown with bacteria and protozoans to be of value. As controls for each core, five plates of media were used to each of which was added the contents of a 1 cc. sterile water blank. While these were the actual controls, it will be seen that the ten dishes containing material from the middle and bottom of the core also acted as checks against laboratory contamination.

Table I gives the results of the plating out of the mud samples.

The fungi recovered from the muds by the methods outlined all belonged to genera commonly found in land soils and easily recovered in spore form from the atmosphere. A large majority were species of *Penicillium*, while others belonged to such genera as *Cephalosporium*, *Trichoderma*, *Aspergillus*, *Chætomium*, *Alternaria*, *Cladosporium*, and even *Rhizopus*. It was soon evident that no fungi which could be considered characteristically marine were being recovered by these methods and interest in them, qualitatively at least, was greatly diminished.

A preliminary experiment was also carried out to determine whether such fungi might be associated with decaying phytoplankton. Using a sterilized net, two sterilized jars were filled with a heavy concentration of phytoplankton from Vineyard Sound. The material was distributed equally between two sterilized battery jars, both of which were then placed under running sea water in the laboratory. By allowing the water to flow very gently into one jar (" A ") the mass of diatoms soon rested on the bottom. Since the control jar (" B ") was subjected to a stronger stream, the material was soon washed out. After four days, during which time the phytoplankton had gradually disintegrated, five 1 cc. samples were plated out from the bottom of each of the jars. Five

TABLE I

Showing the depth and number of cores taken from the stations as well as the number of samples used, their origin and the number of colonies of fungi recovered.

Origin of samples			Water above core		Surface of core		Surface of core + 1 cc. H_2O blank		Middle of core + 1 cc. H_2O blank		Bottom of core + 1 cc. H_2O blank		Control dishes	
Station	Depth	No. of cores	No. of samples	Colonies of fungi	No. of samples	Colonies of fungi	No. of samples	Colonies of fungi	No. of samples	Colonies of fungi	No. of samples	Colonies of fungi	No. of dishes	Colonies of fungi
	meters													
1	18.0	3	15	22	5	1	15	49	15	0	15	1	15	1
2	32.7	4	20	12	15	20	20	59	20	1	20	1	20	1
3	163.6	2	10	17 [1]	10	16	10	22	10	0	10	0	10	0
4	220.0	1 [2]	5	8 [3]	—[4]	—	10	11	10	0	10	1	10	0
5	1127.2	1	5	0	—	—	5	0	5	0	5	1	5	0
6	718.0	1	5	0	—	—	5	2	5	3	5	0	5	0
Totals.....		12	60	59	30	37	65	143	65	4	65	4	65	2

[1] Water very muddy and probably represents fungi from the top layer as well as in water above it.
[2] Two cores were collected but one was discarded because of the possibility that contamination had occurred.
[3] Two of these colonies were at the edge of the dish and were probably contaminations.
[4] There was not sufficient material from Stations 4–6 to use pieces of surface mud alone.

dishes of medium alone acted as controls. Sixteen colonies of fungi were recovered from jar " A " which contained the phytoplankton, and none from jar " B " which contained only the running water. The control plates remained sterile. It was apparent in this case, at least, that there existed a definite relationship between the presence of disintegrating phytoplankton and the moulds. Again, as in the muds, the fungi recovered were all common dust-borne species.

Discussion

From the foregoing, which it must be emphasized is a very preliminary investigation intended more to stimulate further inquiry than to arrive at definite conclusions, certain points seem worthy of further consideration.

If truly marine fungi of a filamentous type exist in the marine muds studied and if there is nothing radically wrong with the methods and type of medium employed, then these organisms must be extremely rare. The types of fungi recovered, i.e., species of *Aspergillus, Penicillium, Trichoderma,* etc., have cast doubts on their being concerned to any great extent in disintegrating processes in the sea. Proof of their presence in marine muds does not mean that they are in an active vegetative state and hence, a working factor in the cycle of decomposition. To the writer's mind, large numbers of direct microscopic observations of mycelium in mud seem the only positive method of demonstrating this important point. The failure of the water cultures to produce saprophytic fungi is also in line with the negative aspects of the data. However, the scanty evidence afforded by the plates of disintegrating phytoplankton presents a more positive picture of relationship. Furthermore, in direct microscopic observations on this disintegrating algal material where freedom from opaque, inorganic matter greatly facilitated the search, active septate mycelium was found in several instances.

Since only land and dust-borne fungi have been recovered it is natural to ask whether they are only contaminants or whether they are actually in the mud. In the collection of the cores every precaution against outside contamination possible with the apparatus was employed and before using the cores the mud surfaces in contact with the tube and the water were cut away. Contamination during culture procedure does not seem likely since mud from the middle and bottom of the cores yielded under identical laboratory manipulation a total of eight colonies in 130 samples and the 65 control plates two. When we consider that 239 colonies developed in the 155 dishes containing samples of supernatant water or mud from the top layer of the core it would seem fairly conclusive that the fungi recovered were actually present in the mud.

Two methods whereby the moulds may have reached the muds are suggested. Either they have been washed from land soils into the sea, or they have been blown, in spore form, from land onto the surface of the water where they have gradually sunk to the bottom. While the stations studied were not in the littoral zone, none was sufficiently offshore to be free from the possibility of obtaining spores from sedimentary material of land origin. Spores are known to be everywhere in the lower atmosphere, and in the immediate vicinity of a continent would be particularly abundant. Many of these would eventually reach the surface of the sea where they might ultimately sink to the bottom. In this connection, qualitative studies (unpublished) by Miss Lois Lillick of the phytoplankton of Vineyard Sound and the Gulf of Maine show that in the former locality fungous spores occur generally throughout the year in the samples, and that they also occur in the shallower waters of the Gulf of Maine, particularly during April and May. It is entirely possible, therefore, that either or both methods of conveyance have contributed fungous material to the marine muds.

Perhaps the most interesting feature of the present investigation has been the information added to our knowledge of the vertical distribution of these ubiquitous moulds. Many types have been recovered from the atmosphere both over land masses and the ocean and even in the stratosphere. Land soils and fresh water have yielded fungi and now they have apparently been recovered in some viable form from the surface of the ocean floor at depths up to 220 meters. Such hardihood is not surprising to those who have observed the capacity of certain of these fungi to withstand adverse environmental circumstances and even to produce under these conditions active vegetative growth.

Finally, it might be emphasized that while no typically marine fungi were found there is strong evidence for believing that certain soil and dust-borne fungi can exist in the surface muds although it has not been shown in what form they occur or that they take an active part there in the disintegration of organic materials.

Summary

Stratified samples of marine bottom were collected under as sterile conditions as possible from four stations which varied from 18 to 220 meters in depth. These stations were located in Buzzard's Bay, Vineyard Sound, and the Gulf of Maine and varied considerably in their distances from land. Attempts were made to recover saprophytic fungi from these cores. Two methods were used: (1) water cultures "baited" with suitable material of marine origin; and (2) the plating out of samples from (*a*) the water immediately above the surface of

the mud core, (*b*) the surface of the core, (*c*) the middle, and (*d*) the bottom of the core. Suitable controls were maintained. No fungi were found in the water cultures. By the plate method using a nutrient medium made up in sea water, 239 colonies were formed in dishes containing water from just above the surface of the core and from the surface of the core itself, eight colonies, in dishes containing material from the middle and bottom of the core, and two colonies in the controls.

A preliminary experiment to determine whether or not fungi were associated with decaying phytoplankton showed definitely that such was the case.

The fungi obtained by the plate method were all common dust and wind-borne forms. Since the methods used in the recovery of fungi did not show in what form they existed on the sea-bottom and since no species which might be called typically marine were recovered, it is doubtful in the present state of our knowledge whether these organisms play an active part in the disintegration of organic materials present in the mud.

BIBLIOGRAPHY

MOORE, H. B., AND R. J. NEILL, 1930. An instrument for sampling marine muds. *Jour. Mar. Biol. Ass'n.*, **16**: 589.

PETERSEN, HENNING E., 1905. Contributions à la connaissance des Phycomycètes marins (Chytridineæ Fischer). *Oversigt K. danske vidensk. Selsk. Forhandl.*, **1905** (5) : 439.

SPARROW, F. K., JR., 1934. Observations on marine Phycomycetes collected in Denmark. *Dansk. bot. Ark.*, **8** (6) : 1.

SPARROW, F. K., JR., 1936. Biological observations on the marine fungi of Woods Hole Waters. *Biol. Bull.*, **52**: 236.

DETERMINATION OF POLARITY BY CENTRIFUGING EGGS OF FUCUS FURCATUS[1]

D. M. WHITAKER

(From the School of Biological Sciences, Stanford University, California)

Introduction

The visible inclusions in the protoplasm of many animal eggs have been segregated within the cell into zones or strata by means of the centrifuge. In smaller, less yolky eggs in which the protoplasm is quite fluid, the inclusions, such as yolk and oil droplets, pigment granules, mitochondria (*Arbacia,* Harvey, 1936) etc., segregate in the protoplasm in accordance with their relative densities. In other cases there is sometimes a rotation or dislocation of regions of the cytoplasm which move as a whole.

The results of numerous earlier experiments on animal eggs are reviewed in such standard texts as Morgan's " Experimental Embryology " (1927) and Wilson's " The Cell in Development and Heredity " (1925), and therefore no exhaustive review of the literature will be undertaken here. It should be noted, however, that in general the mere displacement of the visible inclusions has had surprisingly little effect on the development of most of the eggs. The primary polar axis is not determined by the axis of stratification, although Runnström (1927) has shown that dorso-ventrality may be determined in the sea urchin egg. The general conclusion has been that primary polarity depends on factors in the transparent hyaloplasm rather than upon particles large enough to be moved readily by the centrifugal forces employed.[2] By centrifuging unfertilized *Urechis* eggs at 4,800 × g. for very long periods (up to 18 hours), Taylor (1931) was able to determine or shift the primary polar axis considerably, as shown statistically, but it did not come to coincide precisely with the axis of stratification in most of the eggs. Morgan and Tyler (1935) found that when fertilized *Urechis* eggs are centrifuged, the polar bodies may

[1] This work has been supported in part by funds granted by the Rockefeller Foundation.

[2] Particles moved by the ordinary type of centrifuge may have very pronounced physiological effects, however. Shapiro (1935) has shown that fragments of centrifuged *Arbacia* eggs which contain the heavier granules respire at nearly twice the rate of transparent fragments, and Navez and Harvey (1935) have found twice the indophenol oxidase activity in the fragments containing these granules.

be set free in regions determined by the axis of centrifugation, but in this case the developmental axes of the eggs do not shift with the polar bodies, but instead remain unaltered.

Conklin (1931) found that a dislocation of organs takes place in ascidian eggs when whole regions of the cytoplasm have been displaced or dislocated by centrifuging. Other alterations of the normal development of animal eggs have been brought about by the mechanical consequences of centrifuging, such as twinning (Tyler, 1930; Harvey, 1933, 1935 etc.).

Eggs of the marine brown algæ belonging to the Fucaceæ are more labile than most animal eggs. A number of environmental agents may determine polarity in the *Fucus* egg, including unilateral light (Rosenvinge, 1889) (Kniep, 1907) (Hurd, 1920), especially in the short end of the visible spectrum (Hurd, 1920), direct electric current (Lund, 1923), the presence of neighboring eggs (Rosenvinge, 1889) (Kniep, 1907) (Hurd, 1920) (Whitaker, 1931), especially in acidified sea water (Whitaker, 1937), a pH gradient (Whitaker, 1935) and a temperature gradient (Lowrance, 1937). Knapp (1931) believes that the entrance point of the sperm determines the point of rhizoid origin and the polarity in the egg of *Cystosira,* but the effect of such environmental agents as directed light, if applied at an appropriate time, will supplant this determination and establish a new polarity. The effect of the entrance point of the sperm has not been tested in *Fucus,* but if it determines polarity, this is readily altered by such means as those just indicated. Knapp found further in *Cystosira* that rhizoids form centrifugally when the eggs are centrifuged either before or after fertilization. Schechter (1934) found that rhizoids develop toward the positive pole when pieces of the red alga *Griffithsia* are reared in a direct electric current. Lund (1923) earlier had found that *Fucus* eggs form rhizoids toward the positive pole. Schechter observed that chromatophores migrated toward the positive pole in the *Griffithsia,* and he tried moving the chromatophores by means of the centrifuge (1934, 1935). The chromatophores and other bodies were moved, and accumulated centrifugally under relatively low centrifugal force ($150 \times$ g.) so that the cell-materials stratified, but the place of rhizoid origin was unaffected. Polarity was altered, however, in that shoots formed in the regions to which heavier materials had been thrown.

Method and Results

Eggs of *Fucus furcatus* were obtained from the same locality and by methods which have been described previously (Whitaker, 1936).

Experiments were performed in February and March, 1937. Since this species of *Fucus* is hermaphroditic, and sheds egg and antheridial capsules at the same time,[3] fertilization takes place when the egg capsules break down. This can readily be observed, and eggs were used from capsules breaking down during a time-span of 20 minutes or less. The mid-point of this time-span was counted as the average time of fertilization. The eggs were centrifuged at 3,000 × g. An International electric centrifuge was used which tended to warm up during a 20-minute run, and therefore the sea water in the centrifuge tube and the water around the tube were cooled at the start. The temperature of the eggs during the centrifuging rose from about 8° or 10° to 20° C. At all other times the eggs were kept in a humid, dark, constant temperature room at 15 ± ¼° C. After being centrifuged they were briefly exposed only to red light which does not affect the polarity. The pH of the sea water used in the experiments ranged from 7.9 to 8.0, as measured by means of a glass electrode. In order to rule out the effects of neighboring eggs on each other, no egg lying within 5 egg diameters of another was counted in the results (see Whitaker, 1937).

The First Series of Experiments

In the first series of experiments, the eggs were centrifuged in sea water beginning from 12 to 26 minutes after the average time of fertilization, and were then reared in sea water in Petri dishes. Preliminary survey showed that after being centrifuged for 5 minutes the eggs were quite definitely stratified, but not so sharply so as after being centrifuged for 15 to 20 minutes (see Fig. 1, *A*). It was also found, when the eggs were observed about 24 hours later, that the stratified material had redistributed much more markedly if the eggs were centrifuged for only 5 minutes. In all of the experiments to be cited, the eggs were centrifuged at 3,000 × g. for either 15 or 20 minutes (usually 20). Most of the eggs remained spherical and nearly all of those that were distorted by neighbors in the centrifuge tube rounded up again in the Petri dish.

Figure 1, *A* shows a typical egg 23 minutes after being centrifuged for 20 minutes. Three principal zones are sharply demarcated. At the centripetal end, to which the least dense materials are thrown, there is a cap of globules which presumably are oil or fat. Next to this is a dark brown zone in which the chloroplasts are concentrated, and within which the nucleus is concealed. The remainder of the egg, including

[3] Most of the antheridial capsules dissolve first so that antherazoids, or sperm, are swimming about the egg capsules when they dissolve and set the eggs free in the sea water.

the centrifugal pole, is essentially transparent, although a few plastids may remain scattered especially in the cortical region, and the general texture appears slightly granular. Figure 1, *B* shows the same egg 15 minutes later, and material of the dark zone is already seen to be moving back toward the center of the egg. Soon afterward the nucleus migrates out of the dark band, moving toward the center of the egg. It is usually

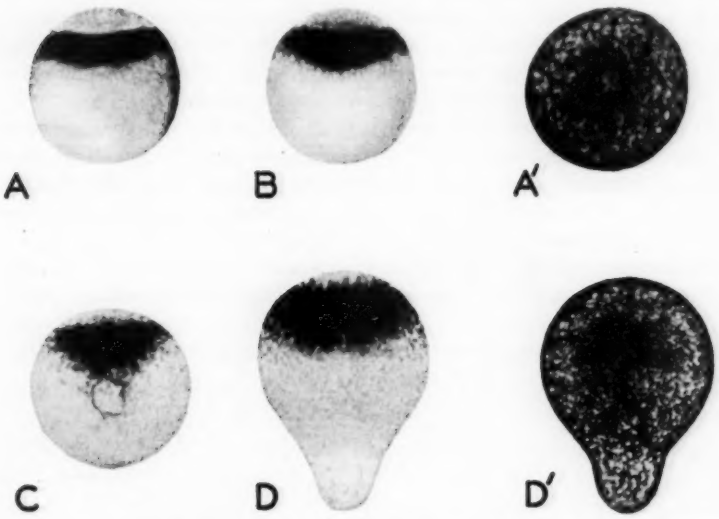


FIG. 1. Photomicrographs of typical developing centrifuged and normal *Fucus* eggs. (*A*) shows an egg 23 minutes after being centrifuged for 20 minutes at 3,000 × g. The centrifuging began shortly after fertilization, and the centrifugal end is below. Chloroplasts are concentrated in the dark centripetal band. (*B*) shows the same egg 15 minutes later, and already inclusions in the dark band are seen to be diffusing back to some extent. (*C*) shows another egg from the same sample 3 hours and 10 minutes later. The nucleus has emerged from the dark stratum within which it was earlier concealed. (*D*) shows another egg from the same sample 13 hours and 25 minutes later. It may be seen, especially by referring to the remnant of the centripetal cap, that the rhizoid protuberance has formed at the centrifugal end of the egg. (*A′*) and (*D′*) are normal non-centrifuged eggs in stages corresponding to (*A*) and (*D*) respectively. All eggs were reared in the dark at 15° C. at pH 7.9–8.0 (see text).

closely followed, and sometimes it is flanked along the sides or surrounded, by redistributing dark material. Figures 1 *C* and 1 *D* show other eggs from the same dish at later times. In Fig. 1, *D* it can be seen by referring to the reduced remnant of the oil cap that the rhizoid has formed quite precisely at the centrifugal pole. Some of the eggs

do not remain stratified but redistribute completely. These will be referred to later.

In six similar experiments of the first series, large numbers of eggs were reared in Petri dishes after being removed from the centrifuge, and the final records were taken 20 to 24 hours later. When centrifuged eggs are placed in a dish of sea water, most of them rotate and lie so that the heavy side is down. When the rhizoid extends downward it lifts the cell body which usually rolls over to one side. The strata can then be seen. Each egg was further rolled over by hand with a glass needle if necessary to observe the stratification when the results were recorded.

Sample counts of over 1,000 eggs indicated that the vast majority of the eggs which remained stratified, so that the axis of centrifugation could be identified, formed rhizoids at or near the ends to which heavier materials had been thrown. In three experiments more precise estimates were made of the positions of the rhizoids. A random count of 490 eggs showed that 96 per cent formed rhizoids within 10° of the centrifugal pole, and the remaining 4 per cent formed them between 10° and 22° from the pole. In addition to the eggs which remained stratified (Fig. 1, *D*), and could be analyzed as above, some eggs redistributed the stratified materials so completely that the axis of centrifugation could not be identified. The proportion of these was usually small, about 4 to 10 per cent, although in one set of eggs, for reasons which are not clear, approximately 50 per cent of the eggs had redistributed at 24 hours. There was usually a sharp distinction between eggs that remained clearly stratified and those that had completely redistributed, with relatively few transitional cases.

From the experiments cited above, it was concluded that the rhizoid forms at the end of the egg to which heavier materials are thrown, at least in the eggs which remain stratified after 24 hours. The *Fucus* egg is spherical and has no early identifying mark of polarity (Fig. 1, *A'*). It is conceivable, however, that the egg actually has a predetermined rhizoid pole which is also the heaviest part of the egg. If so, in centrifuging, the egg would be thrown to the bottom of the tube in an oriented position. This does not appear very probable, but if it were true the stratification would conform to the oriented position and might itself be irrelevant in the determination of polarity. A second series of experiments was therefore designed to test this and other points.

The Second Series of Experiments

In the second series, eggs were embedded in 1.5 per cent agar-sea water before centrifuging, after centrifuging, and without being centri-

fuged. Eggs develop normally in this medium, and observations on stratified eggs in various positions indicate that they are firmly held and that few if any move or rotate within the agar-sea water after it has cooled and jelled, if it is undisturbed. If the agar is cut with a razor, eggs lying close to the cut may rotate as a consequence of the mechanical disturbance. One and a half per cent agar-sea water is a firm jelly at 15° C., and the surface of a fertilized *Fucus* egg attaches to it firmly.

Eggs were embedded by pipetting a concentrated mass of eggs in a minimum of sea water into agar-sea water at 38° C. The agar-sea water gelates below this temperature. The mixture was taken in and out of the agar pipette once or twice to assure mixing and the dish was put on ice to cool rapidly and shorten the exposure of the eggs to high temperature.

When a population of fertilized eggs which are not centrifuged is embedded in agar-sea water and reared in the dark, the rhizoids form at random in all directions as seen from above. The upward and downward components are not equal, however, when the population is considered statistically. Rhizoids are found in every possible position, from straight down to straight up, but more are found with a downward component than with an upward component. The average position is somewhat downward from the horizontal plane. For example, in a population of 413 eggs, 14 per cent of the rhizoids formed upward ($\pm$ 45°), 41 per cent to the side ($\pm$ 45°), and 45 per cent downward ($\pm$ 45°). In other populations more rhizoids formed to the side than downward. It has been shown elsewhere (Whitaker, 1937) that the downward component increases with acidity of the sea water and with increased concentration of eggs. It cannot be said at the present time whether the downward component is due to gravity or to concentration gradients of substances (e.g. CO_2) which diffuse through the upper boundary of the medium but cannot diffuse through the bottom of the dish.

When eggs were first centrifuged, and then mixed at random into agar-sea water, it was found by analyzing 600 eggs statistically after the agar had solidified that the eggs tended to be oriented with the heavy side downward. This orientation, while appreciable, was on the whole not pronounced. It presumably took place just before the agar solidified, as a result of the strong density gradients which had been created in the eggs by stratification in the centrifuge. The results from these eggs which bear on the determination of polarity will be considered after the principal results of the second series have been taken up. These are derived from eggs first embedded in agar-sea water and then centrifuged.

In six similar experiments of the second series, eggs were embedded

at random soon after fertilization in 1.5 per cent agar-sea water in moulds which formed castings which fitted the bottoms of the centrifuge tubes. The castings were centrifuged, and then small blocks or strips of agar, bearing stratified eggs, were cut with a razor and placed in Petri dishes of sea water. All eggs in a block were oriented the same way with respect to their stratification, except for some eggs, especially those near the edge, which were mechanically disturbed and rolled when the block was cut and handled. These blocks were oriented in three different positions: with the centrifugal side (1) straight down, (2) to the side, and (3) straight up. In each experiment, blocks of agar cut from the same centrifuged piece were placed in either two or three of the positions.

After the eggs in these blocks of agar-sea water had developed in the dark for 20 to 24 hours, they were inspected with the microscope and sample counts were made of the positions of rhizoid origin. Whenever necessary, eggs were rolled over within the agar with a glass needle. The eggs were classified as having formed rhizoids downward within 45°, or to the side within 45°, or upward within 45°. The positions of the rhizoids with respect to stratification were noted, and whether or not redistribution had taken place so that stratification was no longer discernible. The results of the separate experiments were essentially similar, and therefore the averaged results will be considered.

Of 423 eggs in agar-sea water blocks placed so that the centrifugal sides of the blocks were downward, 46 or less than 11 per cent had redistributed, while the remaining 89 per cent remained stratified. Ninety-nine per cent of the eggs which remained stratified formed rhizoids within 10° of the centrifugal pole (see Fig. 1, *D*), while 1 per cent formed them farther away from the centrifugal pole, but still on the centrifugal hemisphere. Ninety-five per cent of the eggs which remained stratified had retained their position in the agar, without rotating after being centrifuged, so that their centrifugal poles were downward, while 5 per cent had been rotated so that their centrifugal poles were to the side within 45°. Of the 46 eggs which had redistributed, 3 formed rhizoids downward, 25 to the side, and 18 upward. The distribution of these rhizoids is strikingly different from those on the eggs which remained stratified. Relatively many more are to the side and upward, which suggests either that the determination of rhizoid formation at the centrifugal poles is largely lost when eggs redistribute, or else that a large proportion of the redistributed eggs had been oriented with their centrifugal poles to the side or upward (as a result of rotating when the block was cut), and that such orientations favor redistribution. Evi-

dence that both of these factors operate is provided by eggs reared with their centrifugal poles upward.

Eggs reared in agar-sea water blocks placed so that the centrifugal sides of the blocks were upward were held with the heaviest materials at the top and the lightest at the bottom. This is the exact opposite of the position which the eggs tend to assume when free in a dish, and it should tend to favor redistribution since the effect of gravity is added to that of diffusion. Of 488 eggs which developed in blocks in this position, 166, or 34 per cent had redistributed (compared with 11 per cent in blocks placed centrifugal side downward), and of these redistributed eggs 130 formed rhizoids downward, 33 to the side, and 3 upward. In other words, the percentage of redistribution was trebled, and the redistributed eggs formed rhizoids more nearly in the general pattern found in a population of eggs which have never been centrifuged. Even more rhizoids formed downward than in the average population of non-centrifuged eggs. Three hundred and twenty-two eggs remained stratified, and 93 per cent of these formed rhizoids within 10° of the centrifugal pole. The remaining 7 per cent formed them farther away from the centrifugal pole, but not more than 90°. Two hundred and seventy-two eggs formed rhizoids upward, and 99 per cent of these had remained stratified and formed rhizoids within 10° of the centrifugal pole. Twenty-six of the 488 eggs remained stratified but had been rotated so that the centrifugal pole was to the side ($\pm 45°$), and in 27 it was downward ($\pm 45°$).

Eggs reared in agar-sea water blocks placed so that the centrifugal sides of the blocks were to the side gave results which are on the whole intermediate between those from eggs in blocks in up and in down positions. Four hundred and twenty-five eggs were analyzed. Sixteen per cent had redistributed, compared with 34 and 11 per cent, respectively, in the other two positions. These redistributed eggs formed 1 rhizoid upward, 39 to the side, and 27 downward (all $\pm 45°$). Three hundred and fifty-eight eggs remained stratified and 333 of these (93 per cent) formed rhizoids within 10° of the centrifugal pole. The remaining 7 per cent formed them between 10° and 90° from the centrifugal pole. Of the 333 eggs which formed rhizoids within 10° of the centrifugal pole, 314 (94 per cent) developed rhizoids laterally (i.e., they retained their original orientation in the agar blocks).

As earlier mentioned, eggs were also centrifuged first and then embedded in agar-sea water. The axes of stratification lay at various angles with respect to the horizontal plane. The results obtained by analyzing 600 eggs selected at random were entirely in harmony with those just cited.

Discussion

Most of the centrifuged eggs remain stratified long after the rhizoid protuberances have formed, although the stratified materials begin to diffuse back to some extent quite early (see Fig. 1). The results show that, regardless of the position in which they are held and reared, the centrifuged eggs which remain stratified form rhizoids on their centrifugal hemispheres. With very high incidence, they do so quite precisely at their centrifugal poles to which heavier materials have been thrown. Thus, of 1,057 eggs which remained stratified in blocks of agar-sea water placed so that the centrifugal sides were downward, to the side, or upward (comparable numbers of eggs being in each of these 3 positions), 1,007 or more than 95 per cent formed rhizoids within 10° of their centrifugal poles as marked by the strata. The remainder formed them between 10° and 90° from the centrifugal poles. These eggs were embedded in random positions in firm agar-sea water before being centrifuged, and presumably could not orient in the centrifuge in accordance with any pre-determined polarity. The developmental polarity is therefore determined by the axis of centrifugation. If there is an earlier polarity (as found by Knapp (1931) in *Cystosira;* see introduction), it is completely altered.

Some of the eggs do not remain stratified, but instead the visible inclusions have redistributed so completely when they are observed after the rhizoid protuberances have formed that they are indistinguishable from eggs which have never been centrifuged. When eggs are held inverted in agar-sea water so that the centrifugal poles are uppermost, the proportion of the eggs which redistribute is considerably increased, although the redistributed eggs still remain in the minority. When inverted eggs redistribute, the tendency to form rhizoids at their centrifugal poles disappears. They form rhizoids more nearly in the positions in which they would have formed them if they had never been centrifuged. In fact, both in the blocks placed centrifugal side upward and in those placed centrifugal side downward, the redistributed eggs formed even more rhizoids on the sides of the eggs away from the centrifugal sides of the agar blocks than non-centrifuged eggs would have been expected to do in their place.

The fact that only some of the eggs redistribute, while most others held in the same position do not, must be attributed to variations in the properties of different eggs in regard to factors such as viscosity, etc., and perhaps to the fact that some of the eggs had been fertilized as much as 20 minutes longer than others at the time of centrifugation.

Knapp (1931) found that rhizoids form at the centrifugal poles of

Cystosira eggs, in high percentages if the eggs have been visibly stratified. The principal results on *Fucus* eggs are thus similar. Schechter's (1934, 1935) results on pieces of the red alga *Griffithsia* are similar in that the location of organs was determined by centrifuging, but in this case shoots, not rhizoids, formed at the centrifugal pole. The place of origin of rhizoids was unaffected.

It is possible that a special rhizoid-forming substance accumulates at the centrifugal pole of the *Fucus* egg as a result of centrifugation. However, since gradients of a number of factors which affect the rate of activities such as respiration, CO_2 production, etc. (e.g. temperature, pH, etc.; see Introduction) determine the point of rhizoid origin in this egg, it is also possible that polarity is determined in the stratified egg by gradients of such activities caused by the asymmetrical distribution of cell inclusions. Moreover, these factors may influence the mitotic figure which in turn may affect the polarity. The present experiments do not provide a basis for choosing between these possibilities.

Summary

1. When eggs of *Fucus furcatus* are centrifuged at 3,000 $\times$ g. for 15 or 20 minutes, beginning between 12 and 37 minutes after fertilization, the visible cell inclusions are thrown centripetally (see Fig. 1).

2. Most of the eggs remain visibly stratified until long after the rhizoid protuberances have formed. Ninety-three to ninety-nine per cent of the eggs which remain stratified form rhizoids quite precisely at the centrifugal pole (within 10°; see Fig. 1), even when they have been embedded in random positions in firm agar-sea water before being centrifuged so that they could not orient in the centrifuge in accordance with any earlier polarity. The remainder also form rhizoids on the centrifugal hemisphere of the egg, but farther removed from the centrifugal pole.

3. In eggs which remain stratified, the place of rhizoid origin and the developmental polarity are therefore determined by the axis of centrifugation. This is true regardless of the position in which the stratified eggs are held in agar-sea water during development. If there is an earlier polarity in the egg it is completely supplanted by the effects of centrifugation.

4. In a smaller proportion of the eggs, the cell inclusions have redistributed so completely when the eggs are inspected after the rhizoid protuberances have formed that, in high contrast to the eggs which remain stratified, they are visibly indistinguishable from eggs which have never been centrifuged. Such redistribution is more prevalent when eggs are reared heavy side up in agar than when they are reared heavy

side down. The determination of rhizoid formation at the centrifugal pole is lost in inverted eggs which have redistributed. Under the conditions of the experiments, the determination therefore appears to correlate with the distribution of visible inclusions.

The author is indebted to Mr. C. W. Clancy for assistance in carrying out the experiments.

BIBLIOGRAPHY

CONKLIN, E. G., 1931. The development of centrifuged eggs of ascidians. *Jour. Exper. Zoöl.*, **60**: 1.

HARVEY, E. B., 1933. Effects of centrifugal force on fertilized eggs of Arbacia punctulata as observed with the centrifuge-microscope. *Biol. Bull.*, **65**: 389.

HARVEY, E. B., 1935. Some surface phenomena in the fertilized sea urchin egg as influenced by centrifugal force. *Biol. Bull.*, **69**: 298.

HARVEY, E. B., 1936. Parthenogenetic merogony or cleavage without nuclei in Arbacia punctulata. *Biol. Bull.*, **71**: 101.

HURD, A. M., 1920. Effect of unilateral monochromatic light and group orientation on the polarity of germinating Fucus spores. *Bot. Gaz.*, **70**: 25.

KNAPP, E., 1931. Entwicklungsphysiologische Untersuchungen an Fucaceen-Eiern. 1. Zur Kenntnis der Polarität der Eier von Cystosira barbata. *Planta* (Abb. E), **14**: 731.

KNIEP, H., 1907. Beiträge zur Keimungs-Physiologie und Biologie von Fucus *Jahrb. wissensch. Bot.*, **44**: 635.

LOWRANCE, E. W., 1937. Determination of polarity in Fucus eggs by temperature gradients. *Proc. Soc. Exper. Biol. and Med.* (in press).

LUND, E. J., 1923. Electrical control of organic polarity in the egg of Fucus. *Bot. Gaz.*, **76**: 288.

MORGAN, T. H., 1927. Experimental Embryology. Columbia University Press, New York.

MORGAN, T. H., AND A. TYLER, 1935. Effects of centrifuging eggs of Urechis before and after fertilization. *Jour. Exper. Zoöl.*, **70**: 301.

NAVEZ, A. E., AND E. B. HARVEY, 1935. Indophenol oxidase activity in intact and fragmented Arbacia eggs (abstract). *Biol. Bull.*, **69**: 342.

ROSENVINGE, M. K. L., 1889. Influence des agents extérieurs sur l'organisation polaire et dorsi-ventrale des plantes. *Rev. Gen. Bot.*, **1**: 53.

RUNNSTRÖM, J., 1927. Experimentelle Bestimmung der Dorso-ventral-achse bei dem Seeigelkeim. *Arkiv. for Zoologi.*, **18**: (No. 4), 1.

SCHECHTER, V., 1934. Electrical control of rhizoid formation in the red alga, Griffithsia bornetiana. *Jour. Gen. Physiol.*, **18**: 1.

SCHECHTER, V., 1935. The effect of centrifuging on the polarity of an alga, Griffithsia bornetiana. *Biol. Bull.*, **68**: 172.

SHAPIRO, H., 1935. The respiration of fragments obtained by centrifuging the egg of the sea urchin, Arbacia punctulata. *Jour. Cell. and Comp. Physiol.*, **6**: 101.

TAYLOR, C. V., 1931. Polarity in normal and centrifuged eggs of Urechis caupo Fisher and MacGinitie. *Physiol. Zoöl.*, **4**: 423.

TYLER, A., 1930. Experimental production of double embryos in annelids and mollusks. *Jour. Exper. Zoöl.*, **57**: 347.

WHITAKER, D. M., 1931. Some observations on the eggs of Fucus and upon their mutual influence in the determination of the developmental axis. *Biol. Bull.*, **61**: 294.

WHITAKER, D. M., 1935. Induction of polarity in Fucus furcatus by a localized concentration of hydrogen ions. *Proc. Soc. Exper. Biol. and Med.*, **33**: 472.

WHITAKER, D. M., 1936. The effect of white light upon the rate of development of the rhizoid protuberance and the first cell division in Fucus furcatus. *Biol. Bull.*, **70**: 100.

WHITAKER, D. M., 1937. The effect of hydrogen ion concentration upon the induction of polarity in Fucus eggs. I. Increased hydrogen ion concentration and the intensity of mutual inductions by neighboring eggs of Fucus furcatus. *Jour. Gen. Physiol.*, **20**: 491.

WILSON, E. B., 1925. The Cell in Development and Heredity. The MacMillan Co., New York.

ON THE ENERGETICS OF DIFFERENTIATION, VI

Comparison of the Temperature Coefficients of the Respiratory Rates of Unfertilized and of Fertilizd Eggs [1]

ALBERT TYLER AND W. D. HUMASON

(From the William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena, California)

The results of these experiments show principally that the temperature coefficients of the rates of respiration are the same for unfertilized eggs as for fertilized eggs over most of the temperature range investigated. They diverge somewhat at the lower temperatures, the fertilized eggs giving higher values.

Theoretical Part

An unfertilized egg is generally considered as a resting cell, presumably being concerned merely with keeping itself alive. It should thus exhibit simply maintenance (basal) metabolism. The maintenance metabolism of an organism is measured by the heat production or the respiration under "resting" conditions. The respiration of an unfertilized egg may then be taken as a measure of its maintenance requirements.

A fertilized egg is also considered as having a maintenance requirement, but in addition there are requirements for processes termed growth and differentiation. It is conceivable that by such an agent as change in temperature, these three processes might be affected differently. But from experiments on the effect of temperature on the rate of development and the rate of respiration of fertilized eggs (Tyler, 1936), this does not appear likely, unless these changes are compensatory. The cited experiments had shown that there is the same total oxygen consumption in reaching a given stage of development at one temperature as at another. Thus the temperature coefficients of rate of respiration and development are the same and there is no temperature within the normal range at which development is accomplished with a minimum of respiration.

It was to be expected, then, that the rate of respiration of unfertilized eggs should give the same temperature coefficients as that of de-

[1] This investigation was supported in part by a grant from the Penrose fund of the American Philosophical Society.

veloping eggs. An investigation of this sort was made by Rubenstein and Gerard (1934) on the sea-urchin egg. They found much higher coefficients for the unfertilized than for the fertilized eggs, the average values for Q_{10} being 4.1 for the former and 1.8 for the latter. An examination of their data shows a number of errors in the calculations which, while not greatly affecting the average values, make their conclusions somewhat less convincing. Considering also the difficulties involved in measuring the respiration of unfertilized eggs, it seemed advisable to repeat these experiments on the sea-urchin and in addition to investigate other forms.

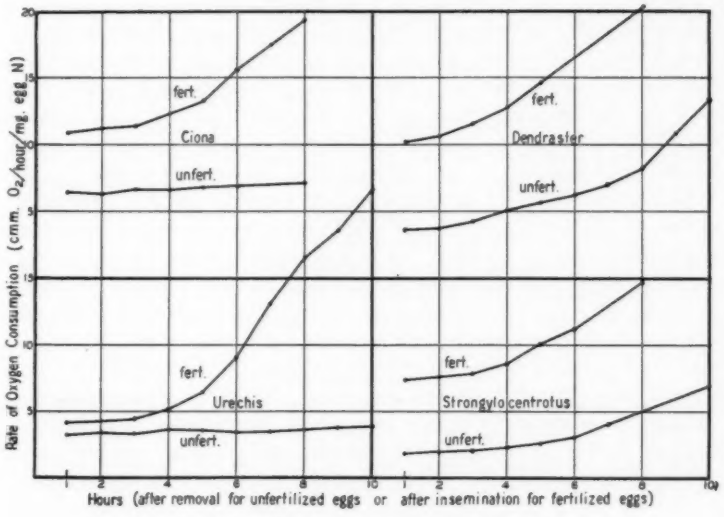
Experimental Part

Material and Methods

The eggs used were those of the sea-urchin, *Strongylocentrotus purpuratus;* the sand-dollar, *Dendraster excentricus;* the echiuroid worm, *Urechis caupo;* and the ascidian, *Ciona intestinalis.*

The manometric method of Warburg was employed for measuring the respiration. Since one of the principal difficulties with unfertilized eggs is their relatively low rate of respiration per unit mass, special vessels were constructed which would tend to make the measured pressure changes as large as possible, at the same time insuring adequate gas exchange. Considering the various factors involved, the cylindrical type of vessel previously described (Tyler, 1936), but with calibration volumes of 18 to 20 cc. and capable of taking 8 cc. of egg suspension, was employed. The vessel constants are of the order of 1.0. The eggs are pipetted into the vessels with special automatic pipettes. Errors in calibration of the vessels and of delivery from the pipettes amount to less than 0.1 and 0.3 per cent and are therefore negligible. The amount of material employed was determined from the nitrogen content of the eggs, obtained by means of a modified Kjeldahl method. The error here depends upon the amount of material employed, but even for the most dilute egg suspension it does not exceed one per cent. In some instances, noted as " no Kjeldahl " in the tables, no nitrogen determinations were made, but the eggs were simply pipetted from a uniform suspension. The manometers are read to the nearest 0.5 mm., so the reading error will be determined by the magnitude of the pressure change. In general no coefficients were calculated for pressure changes of less than 10 mm. and in most cases the readings used were between 30 and 80 mm. In cases in which only dilute suspensions of eggs are available, the runs must be continued over longer periods of time to get sufficient pressure change to reduce the reading error. This involves another difficulty.

One of the chief difficulties that was encountered is the variation in rate of respiration during the progress of a run. The rate of respiration of the unfertilized egg does not remain constant, as is usually assumed, but rises after a shorter or longer period of time. This rise has been previously noted by Warburg (1915) and by Runnström (1930). We find that the unfertilized eggs of the different animals we have studied vary in regard to the rate of rise. This is illustrated in Fig. 1. It



EXPLANATION OF FIGURES

FIG. 1. Rates of oxygen consumption of unfertilized and fertilized eggs of *Ciona, Urechis, Dendraster* and *Strongylocentrotus*. The unfertilized rates for the two latter rise much more rapidly with time than for the other two.

may be seen that the eggs of *Dendraster* and *Strongylocentrotus* show a much more rapid rise than do the eggs of *Ciona* and *Urechis*. In fact, in *Dendraster* and in *Strongylocentrotus* the unfertilized egg respiration rate rises almost as rapidly as does the fertilized egg respiration rate. This rise in respiratory rate appears to be correlated with the loss of fertilizability of the eggs. Without presenting quantitative data at this time, it may be pointed out that *Ciona* and *Urechis* eggs remain fertilizable after standing 24 to 48 hours in sea water whereas *Dendraster* and *Strongylocentrotus* eggs are no longer fertilizable after 5 to 8 hours. Different batches of eggs differ somewhat in the rate of rise, but the

curves of Fig. 1 are typical. The unfertilized eggs of all the forms investigated show sooner or later a rise in respiratory rate.[2]

Occasionally in some runs (e.g. Experiment XI of Table I) a decrease in rate is manifested in the early part of a run. This appears to be due to the sticking of the unfertilized eggs to the walls of the vessels. In some cases a considerable amount (perhaps 5 per cent) of the eggs stick to the walls. The stuck eggs are presumably unaffected while under the sea water, but when the shaking is stopped for a reading some of the stuck eggs are left well above the water level in the vessels. Such eggs, if partially or completely cytolyzed, would give an abnormal respiratory rate. In our experiments on cytolyzing eggs with dry ice or with distilled water we find an initially high respiration followed after about an hour by a very low rate. If this holds for the stuck eggs we would expect to get slight decreases in rate as well as slight initial increases, since most of the sticking occurs at the start of a run.

Due to the variations in rate of respiration with time the temperature coefficients cannot be determined simply by placing a vessel first at one temperature then at another. It is necessary in addition to run eggs from the same batch simultaneously at the two temperatures. The experiments were therefore performed in the following manner. Usually, four vessels were prepared from the same batch of eggs as soon as possible after removal from the animal and two of them were placed in each of two different temperature baths. After a certain number of readings were made the vessels in the high temperature bath were exchanged with those in the low temperature bath. After another set of readings, the vessels were replaced in the original baths and the readings continued. At least fifteen minutes was allowed for temperature equilibrium to be attained.

The temperature coefficients were then calculated in two ways; first, from the oxygen consumption in the vessels run simultaneously at the two temperatures; secondly, from the oxygen consumption in one vessel run alternately at the different temperatures. For the first type of coefficient it is important to know the quantities of eggs in the different vessels or to have identical samples of a uniform suspension in each. For the coefficients calculated in the second manner this is, of

[2] The rise is not due to conditions in the respiration vessels, as an experiment with *Urechis* illustrates. Two samples of eggs, run continuously for 20 hours at 22°, gave Xo_2's of 3.05 and 3.00 cu. mm. in the first hour, and 5.12 and 5.35 cu. mm. in the twentieth hour. Two more samples of eggs from the same batch, that had stood for twenty hours and washed before using, gave Xo_2's of 4.81 and 5.06 cu. mm. in the first hour. Subsequent fertilization was 85 to 100 per cent. The capacity for fertilization is not lost until after a considerable rise in respiration is manifest. In some experiments 100 per cent fertilization was obtained after an almost two-fold rise in the unfertilized rate.

course, unnecessary. Errors in the determinations of the quantity of eggs therefore do not enter into the coefficients calculated in the second manner. However, variations in rate of respiration with time will significantly affect the coefficients calculated in the second manner. To compensate for this the average of two values is taken. For a vessel starting in the high temperature bath, one value is obtained by dividing the initial rate of oxygen consumption by the rate during the ensuing period in the low temperature bath. The other value is gotten by dividing this same low temperature rate into the rate during the following period in the high bath. Where the rate rises, as it generally does, the first value will be lower than the second but the average of the two will be nearer the true value. For a vessel starting in the low temperature bath the two values are obtained in a similar manner. In this case an increasing rate will make the first value too high and the second too low, but again the average will be nearer the true value. The principle involved is the same as in determining the rest point of a balance.

In the tables, the coefficients calculated in the second manner are not given for each individual vessel, but the average for each pair of temperatures is listed in the line "average for individual vessels." The respiration values from which the coefficients are readily determined are given under the headings X_{O_2}. The probable errors are given in cases in which fifteen or more values are averaged. For this purpose, the average for each vessel, determined as mentioned above, is considered as a single value. The last two columns of the tables give the coefficients determined in the first manner; that is, for oxygen consumption during the same period of time at the two temperatures. Where duplicates are run, the coefficient is the ratio of the average oxygen consumption in the two vessels at each temperature. The mean of these values for each pair of temperatures is given in the line marked "average" in the tables. In determining this average an experiment with only two vessels is weighted one-half (rather than one-quarter); that is, two experiments with two vessels are considered the same as one experiment with four vessels. The probable errors are again given for cases in which fifteen or more values are averaged.

In most of the experiments only unfertilized eggs were run, a considerable number of coefficients for fertilized eggs having been obtained in previous work (Tyler, 1936). These values for fertilized eggs are listed in the lines "average from previously published data" in the tables. In some experiments (e.g. VI, X and XI of Table I) two vessels with fertilized eggs were run along with two vessels with unfertilized eggs from the same batch. The figures in parentheses in the tables are for fertilized eggs. In some experiments (I, V and VI of Table V)

after an unfertilized run, the eggs were inseminated in the vessels and the measurements continued. The fertilized eggs give in general much more consistent values, so relatively fewer experiments, in addition to those previously published, were required. The coefficients for the fertilized eggs were calculated here in the same two ways as for the unfertilized eggs. The values taken from previously published data were obtained in a somewhat different manner (*loc. cit.*) which automatically took into account the rising rate of respiration of the fertilized eggs. However, since the rate rises very slowly at the start and since the methods of calculation also allow for the rising rate, comparable values are obtained for short runs. In prolonged runs, values for fertilized eggs obtained in this manner would tend to deviate because the rate of rise increases somewhat with time.

TABLE I

Eggs of Urechis. X_{O_2} = mm.^{3} O_2 consumed per mg. egg-nitrogen; figures in parentheses are for fertilized eggs, the rest are for unfertilized eggs. Q_{10} = ratio of X_{O_2} values (average X_{O_2} where duplicates are run) for the same time intervals at the two temperatures.

Vessels			*A*	*B*		*C*	*D*		
Experiment No.	Time	Temperature	X_{O_2}	X_{O_2}	Temperature	X_{O_2}	X_{O_2}	Q_{10} unfert.	Q_{10} fert.
	hours	° *C.*			° *C.*				
I	1	22	1.99	1.90	12	0.82	0.83	2.37	
	1	12	0.84	0.91	22	2.09	2.17	2.43	
	1	22	2.43	2.22	12	0.97	0.96	2.41	
II	2	22	6.10	6.00	12	2.30	2.04	2.79	
	2	12	2.54	2.49	22	6.43	6.28	2.53	
	2	22	6.41	6.19	12	2.19	2.04	2.98	
III	2	22	4.35	4.24	12	1.79	1.80	2.39	
	1	12	1.07	1.08	22	2.35	2.30	2.17	
	2	22	4.62	4.32	12	2.02	2.05	2.20	
IV	2	22	4.77	4.60	12	2.02	2.05	2.30	
	2	12	2.34	2.22	22	5.03	5.09	2.22	
	2	22	6.24	6.00	12	2.66	2.54	2.35	
V	1	22	2.51	2.43	12	0.89	0.91	2.75	
	1	12	1.01	0.95	22	2.62	2.59	2.65	
	1	22	2.46	2.48	12	1.00	0.95	2.53	
VI	1	22	1.38	(3.38)	12	0.67	(1.43)	2.06	2.37
	1	12	0.62	(1.32)	22	1.64	(3.38)	2.65	2.56
	1	22	1.64	(3.14)	12	0.87	(1.52)	1.88	2.07

TABLE I (*Continued*)

Vessels			*A*	*B*		*C*	*D*		
Experiment No.	Time	Temperature	X_{O_2}	X_{O_2}	Temperature	X_{O_2}	X_{O_2}	Q_{10} unfert.	Q_{10} fert.
	hours	°C.			°C.				
VII	1	22	3.58	3.35	12	1.28	1.25	2.74	
	3	12	3.87	3.93	22	10.91	11.28	2.85	
	3	22	10.14	10.28	12	3.99	4.17	2.50	
VIII	1	22	4.49		12	1.77		2.54	
	2	12	3.00		22	8.23		2.74	
	3	22	10.87		12	3.35		3.24	
IX	2	22	7.38		12	2.90		2.55	
	2	12	2.58		22	6.27		2.43	
	2	22	6.44		12	2.30		2.80	
X	1	22	4.42	(4.50)	12	1.82	(1.65)	2.43	2.73
	1	12	1.20	(1.70)	22	4.46	(4.75)	3.71	2.79
	1	22	3.69	(4.69)	12	1.82	(1.68)	2.03	2.79
XI	1	22	5.06	(4.75)	12	1.96	(1.62)	2.69	2.93
	1	12	1.55	(1.78)	22	5.12	(4.80)	3.30	2.70
	1	22	4.33	(4.88)	12	1.82	(1.72)	2.39	2.84
Average								2.57 ± 0.03	2.64
Average for Experiments II, V and VII								2.70	
Average for individual vessels								2.57 ± 0.04	2.65
Average for individual vessels, Exp. II, V and VII								2.77	
Average for fertilized eggs from previously published data									2.79 ± 0.02

Samples of the eggs from the vessels were inseminated at the end of each run and gave in most cases 90 to 100 per cent fertilization. The experiments in which it was less are as follows: Table I, I, 85, 75, 90, 90; IV, 50, 55, 85, 85; Table III, II, 70, 85; IX, 80, 70, 90, 85; Table IV, XIV, 45, 75; Table VI, I, 15, 20, 35, 30; II, 10, 10, 50, 20. There is no evident relation between the low fertilization in these cases and values of the corresponding temperature coefficients, as an examination of the tables shows.

It is clear from temperature experiments on biological material that coefficients determined at different parts of the temperature scale are not alike. They generally increase as the temperature range is lowered. In other words, Q_{10} (and even μ of the Arrhenius equation) is not a constant. We must therefore make our comparisons for the same temperature intervals and not use the average of values obtained from all

parts of the temperature scale. We have concentrated on one pair of temperatures in attempting to obtain consistent values and supplemented with fewer experiments at other temperatures.

Urechis caupo

Seventeen sets of experiments were run with the eggs of *Urechis;* eleven at the temperatures 22° and 12°, three at 20° and 10°, two at 18° and 8° and one at 15° and 5°. The experiments at 22° and 12° are listed in Table I. The average of the coefficients for the unfertilized eggs is 2.57 by both methods of calculation. For the fertilized eggs (Experiments VI, X and XI) the average Q_{10}'s are 2.64 and 2.65 respectively by the first and by the second methods of calculation. From previous data the value for fertilized eggs is 2.79. Considering the variation in the individual values, we can only conclude that there is no significant difference between the coefficients for the unfertilized and the fertilized eggs in this temperature range.

Comparison of the first and last values of X_{O_2} in each experiment gives the change in rate of respiration. In some instances (e.g. Experiment IV) the unfertilized rate is greater during the last period than at the start. In other cases (e.g. Experiment II) it is fairly constant and in others (e.g. Experiment IX) it drops somewhat. But since the direction of change and relative magnitude is the same at both temperatures, the Q_{10}'s calculated in the first manner are not very greatly affected. Thus for Experiment IV we have 2.30, 2.22 and 2.35. The coefficients calculated in the second manner also are not greatly affected where the rate changes roughly uniformly. Thus in Experiment IV we have the Q_{10} values 2.36, 2.40, 2.19 and 2.24 for the individual vessels. It would, of course, be better to consider only cases in which there is very little change in rate. Taking Experiments II, V and VII as such, we get average Q_{10}'s of 2.70 and 2.77 which are closer to the values for fertilized eggs. We could not, however, find any criterion, such as the extent of agreement between duplicate vessels, the behaviour of the eggs upon fertilization, etc. that would justify the exclusion of any of the experiments listed.

The agreement between the coefficients calculated in the two ways described simply means that, where the rate of respiration changes, the change is fairly uniform. It does not, of course, measure the accuracy of the values.

The experiments at 20° and 10°, 18° and 8°, and 15° and 5° are listed in Table II. At these temperatures, the unfertilized eggs give

TABLE II

Eggs of Urechis. Same description as Table I.

Vessels			A	B		C	D		
Experiment No.	Time	Temperature	X_{O_2}	X_{O_2}	Temperature	X_{O_2}	X_{O_2}	Q_{10} unfert.	Q_{10} fert.
	hours	*°C.*			*°C.*				
XII	1	20	1.74	1.84	10	0.73	0.68	2.55	
	1	10	0.93	0.88	20	1.99	2.10	2.26	
	1	20	2.20	2.17	10	1.00	1.02	2.16	
XIII	1	20	1.95	1.87	10	0.81	0.84	2.30	
	1	10	0.77	0.74	20	2.03	2.07	2.71	
	1	20	1.89	1.87	10	0.78	0.79	2.40	
XIV (no Kjeldahl)	1	20	32.3	(52.3)	10	12.3	(18.2)	2.63	2.87
	1	10	14.9	(20.4)	20	37.0	(52.0)	2.48	2.55
	1	20	31.8	(54.6)	10	11.9	(16.4)	2.67	3.33
Average								2.46	2.92
Average for individual vessels								2.47	2.82
Average for cleavage data									3.30
XV	2	18	2.01	1.97	8	1.10	1.10	1.81	
	2	18	2.28	2.25	8	1.05	0.90	2.32	
	2	18	2.92	2.80	8	0.83	1.00	3.13	
XVI	1	18	1.42	1.42	8	0.60	0.67	2.24	
	1	8	0.83	0.79	18	1.52	1.48	1.85	
	1	18	1.34	1.28	8	0.73	0.78	1.73	
Average								2.18	
Average for individual vessels								1.94	
XVII (no Kjeldahl)	2	15	46.4	(37.9)	5	16.9	(10.9)	2.75	3.48
	2	5	19.5	(11.2)	15	49.7	(36.0)	2.55	3.23
	2	15	55.5	(37.9)	5	15.5	(11.5)	3.58	3.29
Average								2.96	3.33
Average for individual vessels								2.84	3.30

consistently lower Q_{10}'s than do the fertilized eggs. In only two of the experiments were fertilized eggs run. For 20° and 10° no respiration experiments were available from previous work, but the coefficient for cleavage (3.30) which should be the same as for respiration (*loc. cit.*) is listed. For 18° and 8° and for 15° and 5° the cleavage value is

TABLE III

Eggs of Strongylocentrotus. Same description as Table I.

Vessels			A	B		C	D		
Experiment Number	Time	Temperature	X_{O_2}	X_{O_2}	Temperature	X_{O_2}	X_{O_2}	Q_{10} unfert.	Q_{10} fert.
	hours	*° C.*			*° C.*				
I	2	22	3.39	3.60	12	1.30	1.35	2.63	
	2	12	1.23	1.48	22	3.23	3.24	2.39	
	2	22	3.75	4.20	12	1.46	1.58	2.61	
II	2	22	4.74	(16.4)	12	1.65	(6.8)	2.87	2.42
	2	12	2.03	(7.2)	22	5.23	(21.4)	2.57	2.97
	2	22	5.46	(22.1)	12	2.44	(10.6)	2.24	2.08
III	1	22	30.0	(64.7)	12	11.81	(29.5)	2.54	2.19
(no Kjeldahl)	1	12	13.6	(31.7)	22	32.29	(66.0)	2.37	2.08
	1	22	34.2	(66.4)	12	13.18	(30.1)	2.60	2.21
Average								2.54	2.33
Average for individual vessels								2.53	2.39
IV	2	20	2.61	2.65	10	0.67	0.70	3.84	
	2	10	1.22	1.22	20	2.52	2.95	2.24	
	2	20	2.96	3.23	10	1.33	1.34	2.32	
V	1	20	22.3	(22.7)	10	8.9	(10.9)	2.49	2.08
(no Kjeldahl)	1	10	11.0	(10.9)	20	23.8	(23.2)	2.46	2.14
	1	20	25.4	(23.2)	10	12.8	(13.8)	1.99	1.69
VI	1	20	24.4	(62.9)	10		(24.3)		2.59
(no Kjeldahl)	1	10	10.6	(29.0)	20		(64.4)		2.22
	1	20	26.7	(77.3)	10		(27.5)		2.81
VII	1	20	1.48	1.38	10	0.49	0.51	2.86	
	1	20	1.60	1.53	10	0.48	0.51	3.16	
	1	20	1.60	1.62	10	0.54	0.51	3.07	
VIII	1	20	1.03	1.00	10	0.33	0.30	3.22	
	1	10	0.40	0.42	20	1.20	0.92	2.59	
	1	20	1.09	0.96	10	0.55	0.39	2.18	
IX	1	20	1.24	1.27	10	0.42	0.52	2.67	
	2	10	1.19	1.27	20	2.89	2.88	2.35	
	1	20	1.72	1.82	10	0.65	0.67	2.67	
X	1	20	1.21	1.15	10	0.59	0.33	2.56	
	1	20	1.34	1.28	10	0.78	0.41	2.20	
	1	20	1.41	1.34	10	0.95	0.39	2.05	
Average								2.63 ± 0.06	2.26
Average for individual vessels								2.57 ± 0.05	2.23
Average from previously published data									2.30

not given because *Urechis* eggs do not divide at 5° and only occasionally do so at 8°. By themselves, the experiments at these temperatures cannot be taken to demonstrate a difference between the coefficients for the unfertilized and fertilized eggs. They are, however, consistent with the results on the other forms investigated in giving at the lower temperatures somewhat lower values for the unfertilized eggs.

TABLE IV

Eggs of Strongylocentrotus. Same description as Table I.

Vessels			*A*	*B*		*C*	*D*		
Experiment Number	Time	Temperature	XO_2	XO_2	Temperature	XO_2	XO_2	Q_{10} unfert.	Q_{10} fert.
	hours	*° C.*			*° C.*				
XI	1	18	38.5	(48.2)	8	16.0	(16.7)	2.41	2.88
(no Kjeldahl)	1	8	15.5	(19.5)	18	38.5	(48.6)	2.48	2.50
	1	18	40.7	(55.3)	8	16.5	(19.1)	2.46	2.89
XII	1	18	24.4	(61.3)	8	9.3	(23.9)	2.61	2.56
(no Kjeldahl)	1	8	10.0	(27.8)	18	27.1	(65.6)	2.71	2.37
	1	18	27.7	(73.8)	8	10.0	(25.1)	2.77	2.94
Average								2.58	2.69
Average for individual vessels								2.59	2.57
XIII	1	17.5	19.8	(49.9)	7.5	7.4	(18.6)	2.69	2.68
(no Kjeldahl)	2	7.5	18.3	(38.4)	17.5	47.7	(106.3)	2.60	2.77
	2	17.5	54.6	(122.1)	7.5	20.1	(42.0)	2.72	2.91
Average								2.67	2.79
Average for individual vessels								2.69	2.80
								Q_{15}	Q_{15}
XIV	2	20	37.5	(121.6)	5	10.8	(28.9)	3.47	4.21
(no Kjeldahl)	2	5	12.0	(31.3)	20	44.6	(129.0)	3.72	4.12
	½	20	14.8	(39.9)	5	4.1	(9.2)	3.64	4.32
Average								3.61	4.22
Average for individual vessels								3.74	4.24

It is of some interest to compare the absolute values for the rate of oxygen consumption in different experiments. Considering only the first period in each experiment, we see in Table I that the rate varies from as low as 1.38 cu. mm. O_2 per hour per mg. N, as in Experiment VI, to as high as 5.06 cu. mm. as in Experiment XI. The differences

are roughly correlated with the length of time that the animals were kept in the aquaria before the eggs were used, eggs from freshly collected animals giving higher absolute rates of respiration. Animals from different localities vary, but considering, for example, Experiments VI to XI in which the animals were collected at one time in one locality, the times after collection for numbers XI, VIII, X, IX, VII and VI are ½, 3, 4, 9, 10 and 40 days respectively, and the absolute rates of respiration decrease in just about that order. Eggs from the same animal were used in Experiments VII and XI. We shall not attempt an explanation at this time, but we may point out that *Urechis* does not store its eggs in the ovary (if there is a definitive ovary) but in "nephridial" sacs. Also, it is evident that simply aging the eggs in sea water produces the reverse effect, namely a rise in the absolute rate.

With the fertilized eggs we find no marked differences in the absolute rate of respiration. In addition to the figures of Table I we have ten more values for the absolute rate of oxygen consumption during the first hour after fertilization, all of which lie between 4.2 and 4.5 cu. mm. We find thus in *Urechis* cases in which the rate of respiration rises two or three-fold after fertilization, cases of no change in rate and cases (e.g. Experiment XI) of a decrease in rate. Whitaker (1933) showed that in different species the rate of respiration may rise, fall or remain unchanged after fertilization, the absolute rates for the fertilized eggs tending toward the same level in all. Here we have one animal exhibiting all three types of behavior.

Strongylocentrotus purpuratus

Tables III and IV give the respiration data and temperature coefficients for fourteen experiments with eggs of *Strongylocentrotus*. There are three at 22° and 12°, seven at 20° and 10°, two at 18° and 8°, one at 17.5° and 7.5°, and one at 20° and 5°. In all of them the rate of oxygen consumption of the unfertilized eggs shows a rise during the experiment. The shorter runs (e.g. Experiment III of Table III) show a smaller rise than do the longer runs (e.g. Experiment II).

In all of the experiments the temperature coefficients for the unfertilized eggs are fairly close to those for the fertilized eggs. For the temperatures 22° and 12°, the difference is small. For the temperatures 20° and 10°, it is somewhat greater. But if we omit the high values 3.84 and 3.22 of Experiments IV and VIII, the average Q_{10}, by the first method, is 2.27, which is much closer to the value for the fertilized eggs. An examination of the corresponding respiration figures shows that there is some justification for omitting these values, since, in both these experiments, vessels *C* and *D* give oxygen consumption figures that are evidently too low during the first period.

TABLE V

Eggs of Ciona. Same description as Table I.

Vessels			*A*	*B*		*C*	*D*		
Experiment No.	Time	Temperature	XO_2	XO_2	Temperature	XO_2	XO_2	Q_{10} unfert.	Q_{10} fert.
	hours	°C.			°C.				
I	2	25	16.02	14.84	15	9.32		1.66	
	1	15	4.64	4.04	25	9.77		2.25	
	1	25	8.71	8.28	15	4.76		1.79	
I	2	25	(29.72)	(29.36)	15	(13.57)	(14.01)		2.14
	1	15	(7.37)	(7.63)	25	(14.03)	(14.24)		1.88
	1	25	(19.01)	(19.09)	15	(7.31)	(7.07)		2.65
Average								1.90	2.22
Average for individual vessels								1.92	2.14
Average from previously published data									2.27
	hours								
II	1½	22	11.72	11.61	12	7.35	6.04	1.74	
	1	12	3.25	4.07	22	7.97	7.22	2.08	
	1½	22	9.78	11.28	12	4.19	5.47	2.18	
III	2	22	19.88	21.93	12	10.72	11.03	1.92	
	2	12	8.39	8.34	22	16.88	15.28	1.92	
	2	22	17.64	18.28	12	7.04	7.54	2.46	
IV	1	22	7.43	6.30	12	4.06	3.40	1.84	
	1½	12	3.16	4.55	22	8.89	8.28	2.23	
	1	22	6.29	5.06	12	4.68	4.86	1.19	
V	2	22	13.03	10.75	12	5.85		2.03	
	2	12	7.61	5.92	22	12.85		1.90	
	2	22	13.48	12.30	12	5.86		2.20	
V	2	22	(22.50)	(22.39)	12	(9.64)	(9.89)		2.25
	2	12	(9.93)	(9.56)	22	(20.94)	(20.41)		2.12
	2	22	(25.00)	(25.47)	12	(8.84)	(8.47)		2.92
VI	1	22	7.26	6.42	12	3.62	3.85	1.83	
	1	12	3.77	3.49	22	6.55	6.57	1.81	
	1	22	6.22	6.72	12	3.17	3.15	2.05	
VI	1	22	(9.55)	(9.30)	12	(4.16)	(3.67)		2.41
	1	12	(3.58)	(3.49)	22	(9.82)	(8.97)		2.66
	1	22	(10.90)	(10.04)	12	(4.08)	(3.85)		2.64
Average								1.96 ± 0.05	2.50
Average for individual vessels								1.98 ± 0.06	2.47
Average from previously published data									2.84

For the temperatures 18° and 8° and 17.5° and 7.5° there are also no significant differences between the coefficients for the unfertilized and the fertilized eggs, as the figures in Table IV show. One experiment at 20° and 5° shows some difference which may be significant. The unfertilized eggs give lower coefficients (Q_{15} in this case) than do the fertilized, which is consistent with the results on *Urechis* at the lower temperatures. The coefficients for cleavage which could be obtained more accurately than those for respiratory rate, are not given here because the eggs do not divide at 5° C.

TABLE VI

Eggs of Dendraster. Same description as Table I.

Vessels			*A*	*B*		*C*	*D*		
Experiment No.	Time	Temperature	XO_2	XO_2	Temperature	XO_2	XO_2	Q_{10} (unfert.)	Q_{10} (fert.)
	hours	*° C.*			*° C.*				
I	2	22	6.04	5.98	12	2.19	2.30	2.68	
	2	12	3.46	3.28	22	7.73	8.00	2.34	
	2	22	11.08	11.02	12	3.96	4.08	2.75	
II	2	22	4.40	4.05	12	1.96	2.08	2.09	
	2	12	2.72	2.31	22	6.10	6.19	2.44	
	2	22	10.80	9.51	12	3.39	4.17	2.69	
III	2	22	7.46	7.35	12	3.11	3.07	2.40	
	2	22	10.59	10.13	12	3.55	3.98	2.75	
	2	22	12.56	11.82	12	4.26	4.66	2.74	
IV	2	22	7.83		12	3.01		2.60	
	2	22	12.58		12	4.29		2.93	
Average								2.57 ± 0.04	
Average for individual vessels								2.71	
Average from previously published data									2.80

In the experiments of Tables III and IV marked "no Kjeldahl" the quantities of eggs employed were not determined, so the figures in these experiments cannot be compared with the absolute rates in the others. Also we cannot compare unfertilized and fertilized rates in those experiments, since the suspensions of unfertilized and of fertilized eggs were not of the same concentration. In the other experiments, if we compare the absolute rates of respiration for the unfertilized eggs during the first period of a run, we see no such differences as were obtained with *Urechis*. Here, the eggs used came from freshly collected

animals. Whether keeping *Strongylocentrotus* in an aquarium tank would affect the rate of respiration of the unfertilized eggs was not determined.

Ciona intestinalis

Certain difficulties in handling *Ciona* eggs were previously (1936) pointed out. In addition it may be noted that to secure large quantities of unfertilized eggs it is necessary to use many individuals and to wash the eggs thoroughly before mixing them, in order to remove the sperm that almost unavoidably comes out with the eggs. Since *Ciona* eggs tend to float rather than sink to the bottom of the dish, it generally takes about ten or more washings to remove the sperm.

Six experiments were run with eggs of *Ciona*, one at 25° and 15° and five at 22° and 12°. The results are given in Table V. At the temperatures 25° and 15° there are no significant differences between the Q_{10}'s for the fertilized and the unfertilized eggs. In this experiment the vessels *A*, *B* and *C* were removed at the end of the unfertilized run, the eggs inseminated, and the measurements continued on the fertilized eggs. In addition a fourth vessel, *D*, of freshly inseminated eggs was added. Fertilization was 100 per cent in all. At the temperatures 22° and 12° the average Q_{10}'s are somewhat less for the unfertilized than for the fertilized eggs. Considering the magnitude of the difference in relation to the probable error and the fact that it agrees with *Urechis* and *Strongylocentrotus* in giving at the lower temperatures [3] lower coefficients for the unfertilized eggs, we are inclined to regard it as significant.

Comparison of the oxygen consumption figures for the unfertilized and fertilized eggs shows at most a less than two-fold rise upon fertilization. This is much lower than in the case of the sea-urchin egg. The rise is more of the order of that found with *Nereis* eggs (Whitaker, 1931; Barron, 1932).

Dendraster excentricus

Four sets of experiments were done on *Dendraster* eggs, all of them at 22° and 12° C. The results are presented in Table VI. In none were large enough quantities of eggs obtained to get usable oxygen consumption values in less than two hours at each temperature. *Dendraster* shows a fairly rapid rise with time in the rate of respiration of the unfertilized egg. The rate rises even more rapidly than in the case of *Strongylocentrotus* as Fig. 1 illustrates. In the last two hours of a six-hour run we may have more than twice the oxygen consumption ob-

[3] *Ciona* eggs are adapted to a higher temperature range than the others; they fail to divide below 12° and above 26° C.

tained in the first two hours, as in Experiment II. The temperature coefficients, however, are not particularly affected by the rise. This means simply that the relative rise is about the same at different temperatures.

The average Q_{10}'s for the unfertilized eggs by the two methods of calculation are 2.57 and 2.71 respectively. These are slightly lower than the previously determined value of 2.80 for fertilized eggs. The

TABLE VII

Average temperature coefficients (Q_{10}) for unfertilized and fertilized eggs. Probable errors given where sample consists of 15 or more values. Coefficients under *A* are determined from the respiration of eggs in different vessels run simultaneously at different temperatures. Coefficients under *B* are determined from the respiration of eggs in one vessel run consecutively at different temperatures. Coefficients under *C* taken from previously published data.

	Temperatures	Unfertilized eggs			Fertilized eggs			
		No. of vessels	*A*	*B*	No. of vessels	*A*	*B*	*C*
Strongylocentrotus	22° and 12°	8	2.54	2.53	4	2.33	2.39	
	20° and 10°	23	2.63 ± 0.06	2.57 ± 0.05	4	2.26	2.23	2.30
	18° and 8°	4	2.58	2.59	4	2.69	2.57	
	17.5° and 7.5°	2	2.67	2.69	2	2.79	2.80	
	20° and 5°	2	3.61†	3.74†	2	4.22†	4.24†	
Urechis	22° and 12°	34	2.57 ± 0.03	2.57 ± 0.04	6	2.64	2.65	2.79
	22° and 12°	12*	2.70	2.77				
	20° and 10°	10	2.46	2.47	2	2.92	2.82	3.30
	18° and 8°	8	2.18	1.94				
	15° and 5°	2	2.96	2.84	2	3.33	3.30	
Ciona	25° and 15°	3	1.90	1.92	4	2.22	2.14	2.27
	22° and 12°	19	1.96 ± 0.05	1.98 ± 0.06	8	2.50	2.47	2.84
Dendraster	22° and 12°	14	2.58 ± 0.04	2.71				2.80

* Selected experiments, included in line above.
† Q_{15} values.

difference, however, cannot be taken as significant. The two average values for the unfertilized eggs differ more here than in the previous cases. That is because in two of the experiments (III and IV) coefficients could not be determined by the second method (from individual vessels) since the vessels remained at one temperature throughout the run.

DISCUSSION

The average values of the temperature coefficients for the eggs of the four animals investigated are listed in Table VII. As was pointed out

in considering the individual cases, there are no large differences between the unfertilized and fertilized eggs. At the higher temperatures in each case, there are certainly no significant differences. At the lower temperatures, the consistently lower values for the unfertilized eggs of the different animals incline us to regard the difference as significant. With fertilized eggs, or for that matter most biological material (see Belehradek's review), Q_{10} increases as the temperature interval is lowered. Here, it appears that for the respiration of the unfertilized eggs, Q_{10} remains a constant or increases only slightly at lower temperatures. Thus, with *Strongylocentrotus* we get the values 2.54 at 22° and 12° and 2.68 at 17.5° and 7.5°. With *Urechis* the Q_{10}'s are 2.57 at 22° and 12° and 2.90 at 15° and 5°. We are not, however, particularly concerned here with the constancy of Q_{10}. Any other convenient measure of variation in rate with temperature would serve for comparing unfertilized and fertilized egg respiration.

We had expected to find the same values for unfertilized as for fertilized eggs. At the higher temperatures that appears to be the case. But if we accept the divergence at the lower temperatures as significant, then it would seem that one of the assumptions, upon which this expectation was based, must be wrong. This might well be the assumption that an unfertilized egg is a resting cell exhibiting only maintenance. Other processes besides what we term maintenance may be involved. It would seem important then to determine with certainty whether real differences exist at the lower temperatures. We do not, however, consider it likely that with the present material and methods simply expanding the experiments will improve the data very much. Besides, there now appear to be other ways of getting at the questions involved.

The unfertilized eggs of all the forms investigated exhibit a rising rate of respiration. In *Strongylocentrotus* and *Dendraster* the rate rises much more rapidly than in *Urechis* and *Ciona* (see Fig. 1), the difference being correlated with the time of loss of fertilizability on the part of the eggs. Runnström (1928, footnote p. 4) has likewise noted that the sea-urchin eggs lose their capacity for fertilization after exhibiting a spontaneous rise in respiration. There are several agents that have been reported to prolong the fertilizable life of the egg; namely, cyanide (Loeb, 1912) thyroxin (Carter, 1931), alcohol and dextrose (Whitaker, 1937). It would be of interest to know whether these agents would prevent the rise in respiratory rate of the aging unfertilized egg.[4] Under such conditions it is quite possible that different values for the temperature coefficients would be obtained.

[4] Cyanide and CO suppress this rise according to Runnstrom (1930), but the concomitant fertilization test is not given.

Rubenstein and Gerard (1934) reported in *Arbacia* average values of 4.1 and 1.8 for the Q_{10}'s of unfertilized and fertilized eggs respectively. They point out, then, that as the temperature is increased the rise in respiration that occurs upon fertilization in the sea-urchin egg diminishes. By extrapolation they show that at 32° C. there would be no rise. In the sea-urchin, *Strongylocentrotus,* that we have used, as well as in the three other forms, no such differences in the coefficients are evident. At the lower temperatures, there are possibly significant differences, but in the reverse direction from what the above investigators find. However, if it is assumed that Q_{10} for the fertilized eggs decreases as the temperature rises, while Q_{10} for the unfertilized eggs remains constant, the unfertilized eggs would presently give the higher values. By extrapolation, then, if there is any point to it, we would find that the temperature, at which there would be no rise in respiration upon fertilization in *Strongylocentrotus,* approaches that of boiling sea water.

Summary

1. The effect of temperature on the rate of oxygen consumption of unfertilized and fertilized eggs of *Urechis, Strongylocentrotus, Ciona* and *Dendraster* was investigated.

2. The unfertilized eggs exhibit a rising rate of respiration with time in all four species. The rise is much more rapid in *Strongylocentrotus* and in *Dendraster* than in *Urechis* and in *Ciona.* This rise appears to be correlated with the loss of fertilizability on the part of the eggs.

3. Methods of determining the temperature coefficients in such a way as to take into account the general rise (which is a significant factor in prolonged runs) and other variations are described.

4. Only the temperature coefficients for the same temperature intervals are compared, the respiration being determined at two temperatures in each experiment. With *Urechis* and with *Strongylocentrotus* eggs, experiments were run at temperatures between 22° and 5°; with *Ciona,* between 25° and 12°; and with *Dendraster,* 22° and 12°. The experiments with *Urechis* and *Strongylocentrotus* thus included low temperatures at which the fertilized eggs fail to cleave.

5. No significant differences between the temperature coefficients of the respiratory rate of unfertilized and of fertilized eggs of the four animals investigated are found over most of the temperature range in which development is possible. At the lower temperatures, there are differences that are possibly significant, the unfertilized eggs giving consistently lower values.

6. Comparison of the absolute rates of respiration of the unfertilized and fertilized eggs shows in *Strongylocentrotus* and *Dendraster* the rise

in respiration upon fertilization typical of the echinoids; in *Ciona* a less than two-fold rise is manifest; in *Urechis,* the rate may rise considerably, remain constant or decrease slightly, depending upon the particular batch of eggs employed. Eggs from animals kept some time in captivity give lower unfertilized rates and manifest a rise upon fertilization; eggs from freshly collected animals give higher unfertilized rates and no rise or even a slight decrease upon fertilization.

LITERATURE CITED

BARRON, E. S. G., 1932. Studies on cell metabolism. I. The oxygen consumption of Nereis eggs before and after fertilization. *Biol. Bull.,* **62:** 42.

BELEHRADEK, J., 1935. Temperature and living matter. Protoplasma Monographien, vol. 8.

CARTER, G. S., 1931. Iodine compounds and fertilization. III. The fertilizable life of the eggs of Echinus esculentus and Echinus miliaris. *Jour. Exper. Biol.,* **8:** 194.

LOEB, J., 1912. The Mechanistic Conception of Life. University of Chicago Press.

RUBENSTEIN, B. B., AND R. W. GERARD, 1934. Fertilization and the temperature coefficients of oxygen consumption in eggs of Arbacia punctulata. *Jour. Gen. Physiol.,* **17:** 677.

RUNNSTRÖM, J., 1928. Die Oxydationserhöhung bei der Entwicklungserregung des Seeigeleies. *Arkiv. Zool.,* **20** (Art. 3) : 1.

RUNNSTRÖM, J., 1930. Atmungsmechanismus und Entwicklungserregung bei dem Seeigelei. *Protoplasma,* **10:** 106.

TYLER, A., 1936. On the energetics of differentiation. IV. Comparison of the rates of oxygen consumption and of development at different temperatures of eggs of some marine animals. *Biol. Bull.,* **71:** 82.

WARBURG, O., 1915. Notizen zur Entwicklungsphysiologie des Seeigeleies. *Pflügers Arch.,* **160:** 324.

WHITAKER, D. M., 1931. On the rate of oxygen consumption by fertilized and unfertilized eggs. III. Nereis limbata. *Jour. Gen. Physiol.,* **15:** 191.

WHITAKER, D. M., 1933. On the rate of oxygen consumption by fertilized and unfertilized eggs. V. Comparison and interpretation. *Jour. Gen. Physiol.,* **16:** 497.

WHITAKER, D. M., 1937. Extension of the fertilizable life of unfertilized Urechis eggs by alcohol and by dextrose. *Jour. Exper. Zoöl.,* **75:** 155.

SEXUAL AND ASEXUAL REPRODUCTION IN EUPLANARIA TIGRINA (GIRARD)

ROMAN KENK

(From the Miller School of Biology, University of Virginia, and the Department of Zoölogy, University of Ljubljana, Yugoslavia)

Several authors have reported on the reproduction of *Euplanaria tigrina* (Synon. *Planaria maculata* Leidy), one of the commonest freshwater planarians of North America. It has been known for a long time that this species often reproduces by fission. Curtis (1902), in his valuable paper on the life cycle of this form, also states that, at least in several localities, the animals grow sexually mature and deposit egg capsules in the early summer. Much material on the processes of fission has been gathered by experimental workers, particularly by Child and his co-workers. From these we know that the rate of fission may be controlled by external factors, such as temperature, the amount of food, and chemical properties of the water.

Nevertheless, the relations between the two manners of reproduction, the sexual and the asexual one, are as yet little known. The only extensive observations with regard to this question were undertaken by Curtis on material from the vicinity of Woods Hole, Massachusetts. This author investigated the life history of the species in four different localities through the course of three years and summarized his results as follows: "In some localities the species seems to have reproduced exclusively, so far as the observations go, by fission, in others only by the sexual process, while there are still others where both processes occur at different seasons" (1902, p. 556). In a later communication (Curtis and Schulze, 1924, p. 105) he writes: "It may also be noted that the differing habits of reproduction, originally reported . . . for the *P. maculata* in four different localities near Woods Hole, Massachusetts, have been confirmed by all our subsequent collecting in these localities."

These differences in the manner of reproduction in certain places suggested two possible interpretations: (1) there might be some differences in the physical and/or chemical characteristics of these localities, which influence the life cycles of the animals living there; or (2) there might be more than one physiological race of *Euplanaria tigrina*, showing different habits of reproduction. If the latter be the case,

the manner of reproduction would be determined, chiefly, by internal factors.

It is the purpose of this paper to report on a series of experiments performed to analyze several factors that might control these processes. Most of the work has been carried out at the Miller School of Biology, University of Virginia. I wish to express my indebtedness to the Rockefeller Foundation which made this investigation possible, as well as my sincere gratitude to Professor William A. Kepner and Professor Ivey F. Lewis for the privileges extended to me during my stay at the University of Virginia.

Material and Methods

The animals used in the experiments were collected in four localities: (1) Sinclair's Pond, Park Street, Charlottesville, Virginia; (2) Big Spring, near Kerr's Creek, Rockbridge County, Virginia; (3) Mary's Lake, Naushon Island, Massachusetts; and (4) Pond south of Main Street, behind the Episcopal Church, Falmouth, Massachusetts.

In the experiments on asexual reproduction, the influence of temperature, hydrogen-ion concentration, amount of food, and irradiation with ultra-violet rays upon the rate of fission were investigated. The cultures were run in tap water.

Temperature experiments were performed in three series of cultures: at indoor temperature, low and high temperatures. The low temperature cultures were kept in electric, thermostatically controlled refrigerators, while high temperatures were obtained in simple, electrically heated ovens.

The experiments on the influence of the hydrogen-ion concentration in the culture water were carried out at indoor temperature. In order to keep the pH constant, small quantities (1 : 20) of buffer mixtures were added to the water. The buffers used were mixtures of KH_2PO_4 and NaOH having a pH of 6.4, 7.0, and 7.6, prepared after Clark's formulæ. Their addition to the tap water kept the media at a fairly constant acidity of respectively 6.5, 7.0, and 7.5.

The animals were fed with beef liver at regular intervals; it has been proved by various workers that cultures of fresh-water planarians may be run for years on this food. The liver was freely taken.

Ultra-violet irradiation was carried out by means of a Hanovia mercury-arc lamp kindly placed at the writer's disposal by the Committee on Effects of Radiation, National Research Council. These experiments did not yield any results bearing upon the process of reproduction; a more detailed description of the technique employed may, therefore, be omitted.

Experiments on Asexual Reproduction

For these experiments animals from Sinclair's Pond and from Big Spring were used. Each experimental series comprised at least three cultures containing, at the beginning, 5 to 10 specimens each. When fissions occurred, the posterior pieces were eliminated from the cultures. It is well known that the two pieces derived from fission, the head and the tail piece, behave differently in the regeneration of the missing parts. The head piece does not undergo any considerable change, its prepharyngeal part remains almost the same size, while the posterior end appears to grow out of it; the head piece, therefore, regenerates chiefly by epimorphosis. The tail piece, however, shows decided morphallaxis, i.e. it rearranges its proportions and a large part of the old tissues is directly transformed into parts of the missing prepharyngeal region (*cf.* Curtis, 1902, p. 529). Moreover, in a short time, before the regeneration is completed, the tail piece may redivide spontaneously. In head pieces, the intervals between two fissions are more regular. We may, with the necessary caution, take these intervals as indicative of the rate of reproduction. We must, however, realize that an absolute regularity in fission cannot be assumed. Although we select specimens of the same origin, the same age, the same size, and keep them in the same aquarium, yet a great variety of fission intervals will be observed. This is obvious in view of the fact that we cannot control all the factors concerned in the induction of fission. Among these, the nutrition of the individual specimens and their locomotory activity play important rôles. To eliminate the fluctuations of the fission intervals as much as possible, a sufficient number of specimens should be used for each experimental series and the cultures maintained for a sufficiently long time.

In order to express the frequency of fission in a convenient way, two data were calculated for each experimental series: the average interval between two consecutive fissions, and the average fission rate. The latter is the number of fissions per day, calculated for a lot of 100 specimens. It is a disagreeable fact that, in cultures running for a long period of time, the numbers of specimens can hardly be maintained constant, despite careful handling of the animals. The animals are apt to crawl up the wet walls of the culture dishes and then dry up if not noticed in time. Occasionally, individual specimens are lost while one is feeding and changing water. Therefore, the fission intervals and the fission rates were determined for shorter periods (usually 10 days) and from these data the average intervals and rates calculated for the entire length of observation.

Temperature Experiments

The indoor temperature varied considerably during the course of the experiments. In general, it fluctuated between 20 and 30° C., occasionally, for a short time, dropping below these limits (minimum 13.8° C., March 7, 1932) or rising above them (maximum 32.6° C., August 8, 1932). The results obtained in parallel cultures at indoor temperature and in the refrigerator are shown in Table I. At low temperatures (10–12° C.) the processes of fission were greatly inhibited or even entirely suppressed for several months. In a culture of 20 specimens (the number decreased, during the experiment, to 18) running 330 days, only five fissions occurred, the first of them on the 267th day. This shows that at least 13 specimens for almost 11 months

TABLE I

Low-temperature experiments. Average fission rate and fission interval in cultures kept in the refrigerator (10–12° C.) compared with those of control cultures at indoor temperature.

Origin of material	Number of specimens		Temperature	Duration of experiment	Average fission rate	Average fission interval
	Initial	Final				
				days		*days*
Sinclair's Pond	20	18	Refrigerator	330	.084	1189
Sinclair's Pond	15	4	Indoor	341	5.9	16.9
Big Spring	18	18	Refrigerator	329	.15	658
Big Spring	18	2	Indoor	329	7.2	13.9
Big Spring	15	14	Refrigerator	174	.041	2436
Big Spring	15	11	Indoor	174	6.0	16.7

did not display fission at all. The animals were well fed, so the decrease of the fission rate could not be due to starvation. On the contrary, in the culture referred to, the length of the animals was from 5 to 6 mm. at the beginning of the experiment, while at the end lengths of 16 to 20 mm. were measured. The control animals, raised at indoor temperature, generally remained smaller, since they divided before attaining the size of the refrigerator specimens. Another factor that might be suspected of preventing fission is the decreased locomotory activity in the refrigerator culture. The stimulating effect of light was eliminated in both series of cultures by keeping the aquaria in the dark. There is, however, a decrease of the activity caused directly by the low temperature. Nevertheless, since the animals in the refrigerator were often seen to move about in the aquaria, this difference

can hardly be responsible for the extreme rarity of divisions at low temperature.

Several cultures were run at high temperature which varied between 29 and 34.5° C., in the main amounting to about 32° C. They showed a significant change in fission frequency during the course of the experiment (Table II). At the beginning, the fission rates were

TABLE II

High-temperature experiments. Fission rate in cultures kept in the thermostat (about 32° C.), compared with those in control cultures at indoor temperature.

Origin of material	Number of specimens		Temperature	Fission rate		
	Initial	Final		1st–18th day	19th–36th day	37th–55th day
Big Spring.....	14	8	Thermost.	4.8	2.8	.81
Big Spring.....	12	11	Indoor	4.2	2.3	4.3

similar to those of the control cultures at indoor temperature. Soon the divisions became rarer and finally, after 43 days, no fissions occurred any more. Parallel with this decline in reproduction was a reduction of the size of the animals. The lengths of the specimens decreased from 7–13 mm. at the beginning to 3–5 mm. when the experiment was discontinued (after 55 days).

Unfortunately, I had no opportunity to determine exactly the optimum temperature for asexual reproduction. Nevertheless, observations on cultures at indoor temperature indicated that fissions were most frequent when the water temperature was about 25° C. or a little over.

Hydrogen-ion concentration

The observation of fissions in media of different acidity comprised only a small range of pH, viz. 6.5, 7.0, and 7.5. The addition of buffer mixtures to the culture water had no injurious effects on the animals. The reproduction continued in a normal way. No significant differences in the fission rates were observed in cultures of different controlled acidity, nor between them and the cultures in tap water (Table III). It may be noted, however, that the range of pH used was relatively narrow. It is known that planarians tolerate a considerable variation of acidity, towards the acid as well as towards the alkaline side.

TABLE III

pH experiments. Average fission rate and fission interval in buffered culture media and in tap water.

Origin of material	Number of specimens		pH	Duration of experiment	Average fission rate	Average fission interval
	Initial	Final				
				days		*days*
Big Spring	15	11	6.5	168	8.2	12.3
Big Spring	15	13	7.0	168	6.3	15.9
Big Spring	15	10	7.5	174	7.4	13.5
Big Spring	15	11	tap w.	174	6.0	16.7

Irradiation with Ultra-Violet Light

A number of specimens were exposed to the light of a quartz-mercury lamp on several consecutive days. During irradiation, the animals were kept in shallow dishes with little water. The animals so treated showed a slight decrease of the fission rate (Table IV). On

TABLE IV

Fission rates and fission intervals in cultures exposed to different doses of ultra-violet radiation and in control cultures not irradiated.

Origin of material	Number of specimens		Dose	Duration of experiment	Average fission rate	Average fission interval
	Initial	Final				
				days		*days*
Big Spring	15	7	Single	174	4.4	22.8
Big Spring	15	2	Double	174	4.6	21.7
Big Spring	15	11	—	174	6.0	16.7

the other hand, the irradiation apparently exerted a general injurious effect on the animals, which resulted in a high mortality in these cultures. I am, therefore, inclined to attribute the decrease of the fission frequency to the weakening of the animals rather than to a specific effect of the ultra-violet irradiation.

Starvation

It is well known that planarians can stand long periods of starvation very well. In prolonged starvation they grow smaller and, to a certain extent, simplify their anatomical structure. Their physiological condition becomes that of young animals. It has been proved re-

peatedly that starvation causes asexual (as well as sexual) reproduction to cease. This is shown very clearly in Table V. For our experiments, well-fed specimens of a length of 12 to 15 mm. were taken. During the first week of starvation, fissions took place at an almost normal rate; later on, the frequency of division decreased rapidly. The last fission occurred on the twenty-second day after the starting of the experiment. On the sixty-fifth day the culture was discontinued. The size of the animals had decreased to 3½–6 mm.

I wish to point out that no traces of sexual reproduction or maturity were ever observed in any of the cultures of *Euplanaria tigrina* from

TABLE V

Fission rates in cultures starving and in control cultures well fed.

Origin of material	Number of specimens		Nutrition	Fission rate			
	Initial	Final		1st–10th day	10th–20th day	20th–30th day	30th–65th day
Big Spring	30	21	Starving	9.7*	2.4	.3	.0
Big Spring	30	26	Fed	12.0*	4.0	4.3	5.1

* The high fission rates during the first days were due to the fact that fully grown specimens had been selected for the experiment.

Sinclair's Pond and Big Spring, either in the stock aquaria or among the specimens subjected to various external conditions.

EXPERIMENTS ON SEXUAL REPRODUCTION

Since sexuality could not be induced in material from two localities in Virginia, animals were procured from those places where Curtis (1902) had made his observations on the reproduction of the species. In July, 1932, asexual animals were collected in Mary's Lake; at the same time specimens brought in from the Pond in Falmouth proved to be sexually mature and laying egg capsules. Animals from these localities were kept in separate culture dishes, but under external conditions as identical as possible. They were raised at indoor temperature and fed on beef liver.

As a result, the two lots retained, in the main, their characteristic manners of reproduction also in the laboratory. The animals from the asexual locality continued to undergo fission. There were no decided seasonal cycles of reproduction observed, apparently because of the

relatively favorable temperature all the year round. No sex organs developed in the course of 5 years.[1]

The animals from the sexual locality continued to propagate sexually in more or less regular periodical cycles. Though a definite coincidence of the periods of reproductive activity in the laboratory cultures with those observed in the field need not be expected, nevertheless the periodicity conformed, in the main, with Curtis' records of the life history in the natural habitat.

The duration of the egg-laying periods in indoor cultures varied within wide limits. Usually the animals started depositing the first egg capsules in the late fall or in winter (middle of November to February). The breeding reached its height in March and April, then declined, and generally ceased in June. Only once did I observe, in one culture, a breeding season extending from July till the beginning of September. Each specimen deposited several cocoons during one season. The size of the body gradually decreased from about 18–24 mm. to 13–15 mm. After the breeding season was over, the animals were rather sensitive, as if exhausted. The rate of mortality seemed to be higher at that time in the cultures. Nevertheless, most of the animals survived and recovered completely, if well attended to, and in the next egg-laying season again proceeded to produce cocoons. The individual lifetime of this species may be estimated at several years. Curtis' data on the degeneration of the sex organs between two seasons of sexual activity were confirmed.

Temperature Experiments

Like the fission of the asexual form, the rate of breeding in the sexual form is influenced by the temperature of the medium. This is clearly shown in Table VI. At low temperature the laying of cocoons proceeded very slowly; it was almost inhibited; at high temperature the breeding rate increased. On the other hand, the breeding season lasted much longer at low temperatures than at high ones. In the refrigerator culture referred to in Table VI, maintained at 10° C., single egg capsules were deposited at long intervals and the animals remained in the sexual state for at least one year.

[1] One single seeming exception was noticed when, in March, 1933, one sexual specimen appeared in the asexual culture and laid two cocoons. This specimen was subsequently eliminated from the aquarium. Since then and up to now (May, 1937) no second case of sexuality has occurred. I am inclined to assume that this one and only mature animal had, by careless handling, been transferred to this vessel from the sexual culture while the dishes were being cleaned. Even though this assumption were not correct, the occurrence of one sexual individual among many hundreds of asexual animals would not be significant, and could not essentially affect the result of the experiment.

Besides reproduction by cocoons, the animals from the sexual locality also showed asexual reproduction by fission. Fission did not occur in all animals of the cultures and was confined to a short season of the year, from June to August. As this season followed the season of egg-laying, fissions were usually observed in animals which had previously deposited egg capsules. Young animals, hatched from cocoons in the preceding spring, likewise occasionally divided asexually, provided they had already reached a sufficient size and had not yet developed sex organs.

From the fact that the fissioning season coincides with the warmest season of the year, we may conclude that fission requires a high temperature. Temperature, however, is not the sole decisive factor: the animals must, at the same time, be in the state of sexual inactivity. If they are subjected to high temperature during the breeding season, they continue to deposit cocoons and do not divide.

TABLE VI

Temperature experiments on material from the pond at Falmouth. Daily breeding rate (calculated for 100 specimens) and breeding interval in cultures kept at indoor temperature, compared with those kept at lower and higher temperatures.

Temperature	Number of specimens	Duration of observation	Number of cocoons	Average breeding rate	Average breeding interval
		days			*days*
Indoor (about 20° C.).....	20	31	29	4.68	21.4
Refrigerator (about 10° C.)..	19	147	10	.36	279.3
Thermostat (about 28.6° C.)	9	35	98	31.1	3.21

Two tail-pieces of specimens which had fissioned after they had stopped laying egg capsules were studied anatomically. In both of them remainders of the copulatory organs were found, but in a state of apparent degeneration. The genital pore, parts of the atrial cavity, and the penis could still be identified, though their structure differed from that of the organs in function. Bursa and bursa stalk had entirely disappeared. In various places in the parenchyma of the genital region there were patches of brown substance, apparently the product of disintegration of tissues. In short, the pieces exhibited that typical picture of degeneration of sex organs which had been described by Curtis (1902, pp. 546–550) as occurring after the egg-laying.

I should like to emphasize again that fission, in the laboratory cultures, did not occur in the case of every individual specimen. The animals that did undergo fission usually divided only once. After

the tail-piece had grown to a certain size, it occasionally divided a second time if the conditions were still favorable. In any case, fission plays only an insignificant rôle in the life history of the planarian from the pond at Falmouth.

The tendency to divide can be easily controlled by external factors, particularly those that influence the fission rate in the asexual form from Big Spring and Sinclair's Pond, i.e., temperature and nutrition. The following experimental series may illustrate this statement:

April 27, 1936, three cultures of 20 specimens each were prepared. The animals measured 18 to 24 mm. in length and were at the height of the breeding period.

Culture 1. Kept at indoor temperature, fed twice a week with beef liver. Egg-laying ceased June 12, fissioning started June 19 and lasted until August 3. Thirteen animals had divided, 7 remained undivided. These, measured September 21, were from 13 to 15 mm. in length. They entered a new breeding season on March 8, 1937.

Culture 2. Kept at indoor temperature, not fed. The last egg capsule was deposited on May 11; fissions occurred between June 30 and July 22. Only 3 specimens had fissioned, 15 were undivided, 2 had died. September 21, the animals measured 5 to 9 mm. From that time on they were regularly fed. The new breeding period started February 18.

Culture 3. Kept in a refrigerator at 10° C. Cocoons were laid at long intervals,—only 10 cocoons up to September 21, when the animals measured 15 to 21 mm. The egg-laying continued.

It is interesting to note that sexually active animals which had been kept at low temperature for a long time showed an extraordinary increase of the breeding rate when transferred to indoor temperature. This appeared in the continuation of the refrigerator experiment referred to in Table VI. Ten specimens which had been exposed to a temperature of 10° C. for 147 days had shown an average breeding rate of .36 (breeding interval of 279.3 days). Transferred to indoor temperature, they laid during the course of two months no less than 65 cocoons. This corresponds to a daily rate of 10.5 cocoons per hundred specimens or to a breeding interval of 9.5 days. Animals constantly kept at indoor temperature propagate at a much slower rate (see Table VI). From this experiment it would appear that low temperatures, while retarding the deposition of egg capsules at the time, do not inhibit the maturing of the germ cells to the same extent. Brought into normal temperature conditions the animals react to the accumulation of egg cells (and yolk material) in the body by an increased breeding activity.

Discussion and Conclusions

From the foregoing paragraphs it is evident that there must be at least two physiologically different races of *Euplanaria tigrina*. They differ, mainly, in their respective habits of reproduction. We may call them, shortly, the "sexual" and the "asexual" race. The different life cycles of this species in different localities near Woods Hole, Massachusetts, reported first by Curtis (1902 and 1924), are due chiefly to differences inherent in the animals themselves, not to physicochemical properties of the environment. The asexual race lives in Sinclair's Pond, Big Spring, and Mary's Lake; the sexual one in the pond at Falmouth (see p. 281).

Asexual reproduction has been observed in a relatively large number of fresh-water triclads. An excellent review of the material gathered has been given by Vandel (1921, pp. 370–374), who also assumed the existence of different races of *Euplanaria tigrina*, in which the manners of reproduction were hereditarily different. Few observations, however, have so far been made on the entire life cycles of fissioning planarians and little has been done to investigate the processes of reproduction in an experimental way. Nevertheless, it is a striking fact that in almost all species subjected to closer examination, differences in the incidence of sexual and asexual reproduction have been revealed in different localities (*cf.* Benazzi, 1936, p. 364). We know such physiological races in all European species of fissioning planarians: *Polycelis felina* (Dalyell) (Vandel, 1921, pp. 478–479; Thienemann, 1926, pp. 298–300), *Crenobia alpina* (Dana) (Vandel, 1921, pp. 478–479), *Euplanaria gonocephala* (Dugès) (Vandel, 1925; Benazzi, 1936), and *Fonticola vitta* (Dugès) (Beauchamp, 1932, pp. 285–294). Among the American species, sexual and asexual forms are known in *Euplanaria dorotocephala* (Woodworth) (Kenk, 1935*b*, p. 451). The same phenomenon is now confirmed in *Euplanaria tigrina*.

There is no reason to distinguish these races as separate taxonomic units, e.g. species or subspecies. Small morphological differences between them may occur, particularly in the shade of coloration, the size, and the proportions of the body; they are, however, of little taxonomic value. Some of these characteristics, such as the size and shape of the animals, may be directly correlated with the mode of reproduction.

The reproduction of the asexual form of *Euplanaria tigrina* is exclusively asexual. In this the field investigations of Curtis and the experiments described in this paper agree. External factors may accelerate the processes of propagation, retard, or even inhibit them, but they cannot change the process to a sexual one. The main factors

influencing the rate of fission are temperature and nutrition. At low temperatures division is suppressed, it takes place only at about 10° C. or more, provided the animals have attained a certain minimum size. The interval between two fissions is shorter, the higher the temperature. During this interval, the anterior piece at least has to regenerate the posterior portion and therefore the nutrition of the animal plays an important rôle. The optimum temperature, though not exactly established, appears to be between 25 and 28° C. Above this temperature, the fission rate decreases. This decline probably is not the result of a reduced tendency to fission, but a sign of defective nutrition: at high temperatures the metabolism of the body is increased and the food taken does not suffice to cover the energy needs of both maintenance of body size and regenerative growth necessary to prepare the animal for the next fission. The animal is therefore in a state of inanition. This is seen also in the rapid decrease of the size. The maximum temperature tolerated continuously is a little above 30° C. (Lillie and Knowlton, 1898, determined this temperature for "*Planaria torva*" to be 32° C.; the species used in their experiments was probably identical with *Euplanaria tigrina*.)

There appears to be a correlation between the temperature and the size of the body at which fission takes place: at low temperatures the animals do not divide until reaching a considerable length, while at higher temperatures fission occurs already in smaller animals. This seems to apply to all fissioning planarians so far investigated. This phenomenon has not yet been subjected to a comparative study on a larger scale. Castle (1928, p. 420) e.g., observed it in another American planarian, *Fonticola velata*.

In their natural habitats, the life history of the asexual race of *Euplanaria tigrina* is simple: no reproduction in winter, fission during the warmer seasons. This cycle has been observed by Curtis (1902, p. 517) in "locality 4." Moreover, Taliaferro's (1920, p. 63) planarian from an abandoned ice-pond near the University of Virginia, in which reproductive organs were not observed for nine years, apparently belongs to the same race. This applies also to Hyman's (1920, p. 405) material from "the lagoon in Jackson Park in the city of Chicago" (p. 404).

The sexual race of *Euplanaria tigrina*, on the other hand, is capable of both sexual and asexual reproduction. Generally speaking, it develops reproductive organs and deposits egg capsules. Even if conditions be favorable throughout the year, sexual activity is not continuous, but occurs in certain physiological cycles. These cycles, in the field as well as in the laboratory (at indoor temperature),

correspond to the seasons of the year. Apparently the yearly fluctuations of temperature are the decisive factor. The egg-laying season lasts several weeks or months. It goes on until the animals, especially their sex organs, are exhausted. Then follows a period of sexual inactivity during which the complicated copulatory apparatus first degenerates, to be newly reconstructed before the next breeding season. A detailed account of these processes has been given by Curtis (1902).

During the period of sexual inactivity the animals may undergo fission. This occurs only during the warm season of the year and is not at all a regular phenomenon. Many specimens remain undivided.

The regular life cycle can be influenced, particularly by the temperature. Low temperature retards the laying of cocoons, high temperature accelerates it. The formation of egg capsules appears to demand a certain minimum temperature, which lies not far below 10° C.

These data derived from laboratory cultures agree, in the main, with Curtis' observations in the field. Curtis (1902, p. 517) reports that *Euplanaria tigrina* in Locality 1 near Woods Hole, Massachusetts, lays egg capsules during May and June. After that, reproduction ceases and the reproductive organs degenerate. In August and September, i.e. in the season when the water is warmest, the animals are found reproducing by fission. Then reproduction is again suspended and a regeneration of the reproductive organs takes place during the winter.

In Locality 2, according to this author, the life cycle is similar to that in Locality 1, except that no fissions were observed there. There are several possible explanations of this fact: (1) the temperature may not rise so high as in Locality 1, i.e. it may not reach the minimum temperature necessary for fission in the asexual state; (2) the population in this place may belong to a physiologically different race with a fission temperature higher than that of the race from Locality 1 or even with the tendency to fission suppressed; and (3) there may be less food available in Locality 2, wherefore the animals may not reach the necessary size for fission. It would be desirable to investigate this question on the spot.

It is interesting to compare the data concerning *Euplanaria tigrina* with those observed in regard to other fissioning planarians. In cases where both manners of reproduction, the sexual and asexual, alternate according to the seasons of the year, the asexual phase always coincides with the warmer season. The majority of the species concerned develop sex organs preferably in the winter and early spring: *Polycelis felina* (Dalyell) and *Crenobia alpina* (Dana) (according to various

authors); *Euplanaria dorotocephala* (Woodworth) (*cf.* Hyman, 1925, p. 65); *E. gonocephala* f. *subtentaculata* (Draparnaud) (see Draparnaud, 1801, p. 101, and Vandel, 1925, p. 502); *Fonticola morgani* (Stevens and Boring) (see Kenk, 1935*a*, p. 102); *F. velata* (Stringer) (see Castle, 1928, p. 419). In the natural habitats this rule is often obscured by two facts: First, certain localities have an almost constant temperature all the year round, e.g. springs, deep lakes, and subterranean waters; in these habitats often no alternation of reproduction takes place, though the animals in other surroundings would be capable of both sexual and asexual propagation. Secondly, there often occur physiological races, in which the tendency to sexual or asexual reproduction differs to a considerable extent (*cf.* Vandel, 1921, p. 478) and either one of them may be entirely suppressed; these latter forms, of course, are not considered here, since they have no alternation of reproduction.

The sexual race of *Euplanaria tigrina* appears to be different from the other fissioning forms in so far as it becomes sexually active in the warm season of the year. This difference is, however, only apparent. Here, as well as in the others, fission takes place at a higher temperature (August, September) than sexual activity requires (May, June). Only, the minimum temperatures necessary for either kind of reproduction are comparatively high.

It is a matter of further investigation to decide whether this analysis of the processes of reproduction, carried out for two races of *Euplanaria tigrina*, is valid for other forms of this species as well. It may be assumed that additional material will furnish a still greater variety of physiological characteristics.

Summary

1. *Euplanaria tigrina* occurs in at least two physiological races which differ in the manner of reproduction: a sexual and an asexual race.

2. The asexual race, according to observations covering several years, reproduces exclusively by fission. Temperature and nutrition control the rate of fission but do not induce sexuality.

3. The sexual race periodically develops reproductive organs and lays cocoons. After the breeding period has ceased, the sex organs degenerate and fission may occur at high temperature. The individual animal can outlive several periods of sexual activity.

4. In nature, all planarians that have alternating (sexual and asexual) reproduction, appear to propagate sexually during the colder season and asexually during the warmer season of the year.

LITERATURE CITED

BEAUCHAMP, P. DE, 1932. Biospeologica. LVI. Turbellariés, Hirudinées, Branchiobdellidés. Deuxième série. *Arch. Zool. expér. gén.*, **73**: 113.

BENAZZI, M., 1936. Razze fisiologiche di Euplanaria gonocephala differnziate dalla diversa attitudine alla scissiparità. *Rend. R. Accad. Lincei (Roma), Cl. Sc. fis.*, **23**: 361.

CASTLE, W. A., 1928. An experimental and histological study of the life-cycle of Planaria velata. *Jour. exper. Zool.*, **51**: 417.

CURTIS, W. C., 1902. The life history, the normal fission, and the reproductive organs of Planaria maculata. *Proc. Boston Soc. Nat. Hist.*, **30**: 515.

CURTIS, W. C., AND L. M. SCHULZE, 1924. Formative cells of planarians. *Anat. Rec.*, **29**: 105.

DRAPARNAUD, J., 1801. Tableau des Mollusques terrestres et fluviatiles de la France. Montpellier et Paris, (1801).

HYMAN, L. H., 1920. Physiological studies on Planaria. IV. A further study of oxygen consumption during starvation. *Am. Jour. Physiol.*, **53**: 399.

HYMAN, L. H., 1925. The reproductive system and other characters of Planaria dorotocephala Woodworth. *Trans. Am. micros. Soc.*, **44**: 51.

KENK, R., 1935*a*. Studies on Virginian triclads. *Jour. of the Elisha Mitchell Scient. Soc.*, **51**: 79.

KENK, R., 1935*b*. A morphological proof of the existence of zooids in Euplanaria dorotocephala. *Physiol. Zool.*, **8**: 442.

LILLIE, FR. R., AND F. P. KNOWLTON, 1898. On the effect of temperature on the development of animals. *Zool. Bull.*, **1**: 179.

TALIAFERRO, W. H., 1920. Reactions to light in Planaria maculata, with special reference to the function and structure of the eyes. *Jour. exper. Zool.*, **31**: 59.

THIENEMANN, A., 1926. Hydrobiologische Untersuchungen an den kalten Quellen und Bächen der Halbinsel Jasmund auf Rügen. *Arch. Hydrobiol.*, **17**: 221.

VANDEL, A., 1921. Recherches expérimentales sur les modes de reproduction des Planaires triclades paludicoles. *Bull. biol. France Belgique*, **55**: 343.

VANDEL, A., 1925. Planaria subtentaculata Drap. n'est qu'une race asexuée de Planaria gonocephala Dugès. *Bull. biol. France Belgique*, **59**: 498.

INVESTIGATIONS AS TO THE LOCALIZATION OF THE MICROMERE-, THE SKELETON-, AND THE ENTODERM-FORMING MATERIAL IN THE UNFERTILIZED EGG OF ARBACIA PUNCTULATA

SVEN HÖRSTADIUS

(From the Zoötomical Institute, University of Stockholm, and the Marine Biological Laboratory, Woods Hole, Massachusetts)

I. Introduction

The available information about the localization of micromere-, entoderm- and skeleton-forming material, and about the stability of the egg-axis in the uncleaved egg of the sea urchin is not consistent. Driesch (1896, 1898, 1899, 1900) studied the cleavage of fragments (obtained by shaking) of unfertilized and fertilized eggs of *Echinus*. He came to the conclusion that the formation of micromeres is due to the cytoplasm, not to the nucleus. By way of explanation he suggested " ein polar-bilaterales Gerichtetsein der Teilchen " of the whole egg, but he also spoke of a certain local specific structure of the cytoplasm. Morgan (1894) found in part a migration of pigment, in part a formation of micromeres at the vegetative pole of *Arbacia*, even when the furrows were formed in an atypical sequence. Boveri (1901*b*) called attention to the vegetative polar cap and its specific qualities, and Hörstadius (1928, p. 14) confirmed the results of Boveri: vegetative and meridional halves of *Paracentrotus* may show a typical cleavage, whereas the animal half does not form any micromeres. By removing vegetative fragments of different sizes from the unfertilized *Paracentrotus* egg, Hörstadius (*loc. cit.*, p. 18) proved that the potentiality of forming micromeres gradually decreases from the vegetative pole towards the animal and ceases about halfway between the normally micromere-forming area (unpigmented) and the equator. The micromere-forming region is thus restricted to the unpigmented pole-cap and the lower half of the pigment ring material in *Paracentrotus*. (Boveri (1901*b*, p. 155) also saw a pigmented micromere.) Furthermore, Hörstadius (*loc. cit.*, p. 15) found, by aid of the pigment band, that the cleavage axis in fragments remained unchanged, the micromeres both in meridional and vegetative fragments being formed from the unpigmented pole-cap.

Whereas Driesch (1900 etc.) in his early papers, spoke of all parts of

the sea urchin or starfish egg as equipotent, Zoja (1895), Terni (1914), v. Ubisch (1925) and Hörstadius (1928) found that animal halves of cleavage stages could not gastrulate, nor form any skeleton. Boveri (1901*b*, p. 158, 1902) used the pigment ring of *Paracentrotus* to study whether the polarity remains unchanged in fragments of unfertilized eggs. Animal fragments (without pigment) did not gastrulate. One vegetative and one meridional fragment gave larvæ in which the pigmented and unpigmented regions were differentiated in such a way as to show that the polarity of the fragments was not altered. Hörstadius (1928, p. 33) confirmed these results. Animal halves of both fertilized and unfertilized eggs of *Paracentrotus* developed in the same ways as animal halves of 8- or 16-cell stages. They do not gastrulate, nor do they form skeleton. Meridional and vegetative fragments, on the other hand, invaginate archentera and produce spicules. Thus not only the micromere-forming, but also the archenteron- and skeleton-forming material is restricted, in the unfertilized *Paracentrotus* egg, to the vegetative half. Isolation of animal and vegetative fragments of the 64-cell stage in a plane corresponding to the middle of the pigment ring region (between veg_1 and veg_2, Hörstadius, 1935, p. 319) demonstrated that the upper level of the skeleton-forming area corresponds roughly to that of the micromere-forming material in the uncleaved egg. This upper limit of the skeleton material was not determined in detail in the unfertilized egg (*cf.* above as to the micromere-forming material). By aid of the pigment ring Hörstadius (1936*a*) also showed in agreement with Boveri, that the polarity of fragments of the unfertilized eggs does not change.

These investigations on *Paracentrotus* have proved that both the micromere-, entoderm-, and the skeleton-forming material is localized in the vegetative part, roughly speaking, the vegetative quarter, of the unfertilized egg, and that the polarity of the egg remains unchanged in fragments, both as regards the cleavage axis and the differentiation axis.

Some results obtained by other investigators on different material conflict considerably with this view. Harnly (1926) found the cleavage pattern of fragments of unfertilized eggs of *Arbacia* dependent, not upon the orientation of the plane of fragmentation in relation to the egg-axis, but in relation to the nucleus. Boveri (1901*a*) stated that the pronucleus of the mature egg of *Paracentrotus* may have any position in relation to the egg-axis. Harnly (1926) and Hoadley (1934) found the same thing in *Arbacia*. Thus neither the animal, nor the vegetative half is noticeably preferred. From the different cleavage patterns resulting on dividing eggs in different planes with relation to

the nucleus, Harnly comes to the conclusion that the micromere-forming material prior to fertilization lies in the region between the pronucleus and the center of the egg. A nucleated fragment as large as half an egg, or larger, cleaved as does the whole egg. When an egg was divided into two equal halves in the plane shown in Fig. 2, the non-nucleated fragment did not form any micromeres. But when the egg was cut in a plane close to the nucleus, it was the non-nucleated fragment that cleaved as a whole egg. Shortly after fertilization Harnly found a different arrangement. Now only the half containing the egg nucleus will divide. Both this and the uncleaved half remained inside the intact fertilization membrane. Fragments that must have been meridional and vegetative cleaved as whole eggs, and other fragments segmented as animal halves. These results from fertilized eggs agree with those of Boveri and Hörstadius, although Harnly's interpretation is somewhat different (see p. 309),—Harnly studied only the cleavage pattern; he did not follow the further development.

Taylor and Tennent (1924), Taylor, Tennent and Whitaker (1926), and Tennent, Taylor and Whitaker (1929) obtained essentially different results for *Lytechinus* (*Toxopneustes*) *variegatus*. The polar bodies and the funnel (micropyle, made visible by octopus ink) served as landmarks for orientation. These authors state that a new polar axis is established in fragments. With a few exceptions, the first two planes of cleavage were at right-angles to the surface of section, regardless of the orientation of the cut, and the micromeres were formed on the cut side at the end of the intersections of these two planes. Nor is there a complete segregation of the presumptive primary mesenchyme or entoderm in the vegetative part of the mature, unfertilized egg. Their experiments indicated that, prior to fertilization, a mesenchyme-entoderm-forming substance has a uniform distribution through about 19/20 of the egg; the animal polar cap alone seemed to contain only ectoderm material. In the larger part of the egg, however, the presumptive entoderm and mesenchyme was supposed to be restricted to the interior, surrounded by a superficial layer of ectoderm-forming material, as small superficial fragments never gastrulated. Thus both animal, vegetative, meridional and oblique halves of *Lytechinus* could form not only micromeres, but also mesenchyme, and could develop to plutei. In most cases a new polar axis was established, perpendicular to the cut side.

In August, 1936, when spending the summer as a fellow of the Rockefeller Foundation at the Marine Biological Laboratory, Woods Hole, I had the opportunity of testing the localization of the micromere-, the entoderm-, and the skeleton-forming material in the egg of

Arbacia punctulata. Because of the short time available for this investigation the material examined was scanty, but as the results of the observations are very definite, I think they are worth publishing. They indicate that localization in the *Arbacia* egg does not differ from that in *Paracentrotus*.

The eggs were cut with a fine glass needle manipulated by hand. In order that the eggs should not slip away during the sectioning they were placed in a little scratch in a piece of celluloid (Hörstadius, 1928). The cut was orientated either in relation to the pronucleus (Fig. 2), or to the micropyle, which was made visible by ink from the squid (*Loligo*) (Fig. 1).

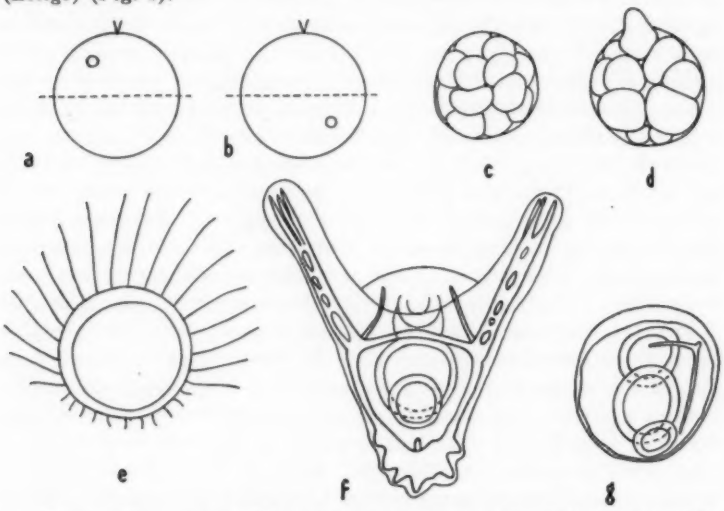


FIG. 1. Cleavage and differentiation of animal and vegetative halves of the unfertilized egg of *Arbacia punctulata*. Orientation by means of the micropyle (*a*, *b*). The egg nucleus may lie in the animal or the vegetative half (*a*, *b*). *c*. Equal cleavage of the animal half. *d*. The vegetative half has formed macro- and micromeres, the latter at the original vegetative pole, antipolar to the cut side, where a cytoplasmic lobe has protruded through a slit in the fertilization membrane. *e*. Differentiation of the animal halves into blastulæ with enlarged apical tuft. *f*, *g*. The vegetative halves gastrulate and form skeleton, developing to ovoid larvæ (*g*) or plutei (*f*). The results indicate a segregation of the micromere-, the entoderm-, and the skeleton-forming material in the vegetative part of the egg.

II. Isolated Animal and Vegetative Halves, and Meridional Halves

Twelves eggs were divided equatorially. At the operation the pronucleus in some cases lay close to the plane of section, in other cases far away from it. In 7 pairs the egg nucleus lay in the animal half, in 5 in the vegetative (Figs. 1*a*, *b*). The animal and the vegeta-

tive halves of each egg were reared separately and kept in pairs. Of the twelve pairs only one vegetative fragment died early.

A. Differentiation

Zoja (1895), Boveri (1901*b*), Terni (1914), and others have stated that animal fragments do not gastrulate and possess a very enlarged apical tuft. This phenomenon was studied in greater detail by Hörstadius (1935, pp. 283–314).

None of the twelve animal halves gastrulated. They all developed considerably enlarged apical tufts, thus differentiating completely in conformity with animal fragments in *Paracentrotus* (Fig. 1*e*). On the other hand, all the vegetative fragments gastrulated and formed skeletons. Some of them developed into good plutei with mouth and arms (Fig. 1*f*), the others showed a more vegetative type with ovoid body shape, no mouth, and a poor skeleton (Hörstadius, 1928, 1935) (Fig. 1*g*).

As controls for the animal and vegetative halves four pairs of meridional halves were also isolated. In two pairs only one partner developed. The other six larvæ (two pairs and two single larvæ) all gastrulated and formed skeletons, developing into more or less typical plutei, or only ovoid larvæ.

B. Cleavage

After fertilization of egg fragments a fertilization membrane forms in the usual way. In *Arbacia* the membrane is not raised from, but lies close to the surface of the egg. When, on cleavage, the volume of the egg increases because of the division into blastomeres, the membrane often bursts on the cut side, and a lobe of cytoplasm protrudes through the opening (Harnly, 1926; Tennent, Taylor and Whitaker, 1929, p. 18; Hörstadius, 1936*b*, p. 820). Sometimes this cytoplasmic bud is nearly cut off by the edges of the membrane, but very often it is connected with the egg by a rather broad base. Nuclei may migrate into the lobe, which may thus be divided into cells. In both cases the bud indicates the cut side.

Of the 12 vegetative halves 11 formed micromeres. Morgan (1894) found that the red pigment in the *Arbacia* egg in the 4-cell, seldom in the 2-cell stage, migrates away from the vegetative pole. As a consequence the micromeres are lighter and whiter than the other blastomeres. The micromeres in our vegetative halves were of this whitish type. But there were not always 4 micromeres (only in seven cases). In one case there was only 1, in two cases 2, and in one case 3 micromeres. One egg showed an irregular cleavage,—it was the vege-

tative half that died. It is a well-known fact that the formation of micromeres may be suppressed, even in an entire egg, without any influence on the later development. Thus the primary mesenchyme is not dependent on the presence of micromeres during the cleavage. The suppression of micromeres is often caused by a slight mechanical disturbance (Boveri, 1901*b*; Hörstadius, 1928, p. 124). The absence of one, two, or three micromeres is probably due to such secondary influences.

The essential point is the relation of the cytoplasmic bud to the micromeres, i.e. the position of the micromeres in relation to the egg-axis. In three eggs no lobe was formed, in all the other eight cases the cytoplasmic bud was antipolar to the micromeres (Fig. 1*d*). This shows, without any exception in the cases where the landmark was visible, that the micromeres were formed at the original vegetative pole.

The cleavage pattern of the meridional halves was not so regular. In one pair both halves had cells of somewhat varying sizes, but no real micromeres were observed. In another pair one fragment had all cells of equal size, those of the other being slightly irregular. In the remaining two pairs, one half had no small cells, the other two either light micromeres close to the bud or only one small cell, the character of which is uncertain. (In the absence of the cytoplasmic bud its position could not be determined.)

Eleven of the 12 animal halves showed cells of equal, or slightly varying sizes, but no micromeres (Fig. 1*c*). The twelfth fragment possessed two pairs of small cells, but they cannot be regarded as real micromeres, as they were not lying close together.

C. Conclusions

In dealing with the often irregular cleavage patterns of fragments, we have to define what is meant by a micromere. Not every small cell is a micromere. Not even every small, whitish cell. As we shall find below, small whitish cells can be formed, owing to particular factors, so that we cannot speak of real micromeres. We only define as micromeres those small, whitish cells which have been formed by a process of fundamentally the same character as in the normal egg.

The isolation of animal and vegetative halves of the unfertilized egg of *Arbacia* gave exactly the same results as with *Paracentrotus*. Animal halves show partial cleavage (no macro- and micromeres are formed, Fig. 1*c*), and differentiate as isolated animal halves typically do, with enlarged apical tuft, and without gastrulation (Fig. 1*e*). The vegetative halves segment as whole eggs, and without any rotation of the egg-axis (Fig. 1*d*). The micromeres are formed at the original

vegetative pole, opposite the cut (equatorial) side, thus not on the cut side as in *Lytechinus*. The vegetative halves of *Arbacia* also differentiate as isolated vegetative halves of other species do, giving ovoid larvæ or plutei (Fig. 1*f*, *g*). This characteristic cleavage and differentiation of animal and vegetative halves takes place irrespective of whether the larvæ are haploid or diploid, and irrespective of the position of the pronucleus of the egg in relation to the plane of section. The results thus indicate a segregation of the micromere-, the entoderm- and the skeleton-forming material in the vegetative part of the egg.

The meridional halves also support this view. All surviving fragments gastrulated and formed spicules. It is important to note that micromeres were not formed very regularly. In the only positive case did they lie close to the cytoplasmic bud, thus close to the cut side, as one would expect. This absence of micromeres is probably due to the fact that the mechanism of micromere formation—especially in the unfertilized egg—is very sensitive to mechanical injury (Hörstadius, 1928, pp. 19, 124), and that the cut passes through the supposed micromere-forming region. In *Paracentrotus* I obtained micromeres also in meridional halves in most cases, but there the membrane is less tight, or perhaps the vegetative cytoplasm less sensitive. It is to be regretted, that more cases were not available for study.

III. Fragments of Equal Size Isolated by a Cut as Far Away from the Nucleus as Possible

To test Harnly's results, unfertilized eggs were divided into two approximately equal halves by a cut as far from the nucleus as possible. Eggs were chosen in which the nucleus lay fairly close to the periphery. The plane of section was perpendicular to a line through the nucleus and the center of the egg and passing through the latter (Fig. 2*a*). All the micromere-forming material which, according to Harnly, lies between the nucleus and the center of the egg, should be found in the fragment containing the egg nucleus. The plane of section thus is at random in relation to the egg-axis. Fifty-one pairs were reared.

A. Differentiation

Not all the fragments started to develop after fertilization; some remained undivided. A few happened to get lost. But in 35 pairs both partners reached full differentiation (Fig. 2). In 13 only one of the two fragments developed. In 18 pairs both fragments gastrulated and formed skeletons, developing into plutei or ovoid larvæ. In 5 the diploid partner differentiated as an animal half (enlarged apical tuft, no invagination), the haploid as a vegetative (archenteron,

skeleton, ovoid larva or pluteus), and in 6 pairs I found the reverse, the haploid fragment behaving as an animal half. In addition there were 3 cases in which the diploid had only a small archenteron, with or without skeleton, but the haploid a typical or large archenteron, and skeleton, whereas in another 3 pairs the reverse was the case (the haploid larvæ with a small invagination—Fig. 2). The 13 single larvæ developed as follows: 7 diploid, 2 haploid larvæ with more or less typical archentera and skeletons; 1 diploid gastrula with too small invagination; 1 diploid and 2 haploid blastulæ with enlarged apical tuft.

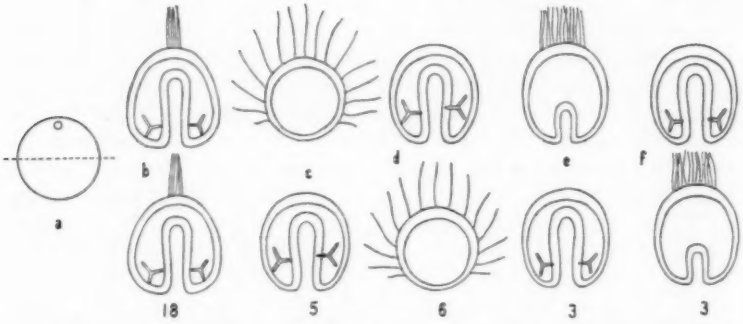


FIG. 2. Diagram of the differentiation of halves of the unfertilized egg of *Arbacia* isolated by a cut perpendicular to the line nucleus-center of the egg. The cut thus at random in relation to the egg-axis. In 18 pairs both partners gastrulated and formed skeleton, developing like approximately meridional halves (*b*). In 11 pairs the one larva differentiated as an animal half, which in 5 of those cases was diploid (*c*), in 6 haploid (*d*). In 3 pairs the diploid, in 3 the haploid fragment developed into a gastrula with too small archenteron (*e, f*). The results demonstrate a localization of entoderm- and skeleton-forming material in a part of the unfertilized egg that must be smaller than half the egg, and independent of the position of the egg nucleus.

B. Cleavage

We have already seen that the cleavages of the meridional halves were somewhat atypical, as the micromere formation was often inhibited. Moreover, the blastomeres may often vary more or less in size. In the halves isolated by a cut at random in relation to the egg-axis, we find all sorts of cleavage patterns.

It has been emphasized above that probably not all small cells observed are micromeres. On the contrary, small cells evidently may be formed as a result of factors fundamentally different from those leading to the formation of the typical micromeres. One has to be very careful regarding the interpretation of the nature of the small cells. Sometimes the cytoplasmic bud, protruding through the opening of the fertilization membrane—although not containing any nuclei

or real cell membranes—may at the first glance look like a micromere, or a group of micromeres. In some cases the cell membranes at one or two divisions may also pass through the cytoplasmic lobe, but the small "cells" formed in that way will still lack nuclei. Very often, however, a nucleus migrates out into the bud and subsequently divides there. After two divisions, the lobe is divided into four small (if the lobe was small) cells, which are similar to micromeres, in that they are lighter than the other cells (the cytoplasmic bud is often not so markedly pigmented as the other part of the egg). When the lobe does not protrude much outside the membrane, these cells have a striking similarity to micromeres. But we can also find small cells, not micromeres, inside the membrane, but formed in connection with the bud. If the cytoplasmic bud is pressed out in the 2- or 4-cell stage, one of the quarter-blastomeres may be much smaller than the other ones. After two further divisions its descendants will constitute four small cells inside the fertilization membrane (or two or three, if one nucleus has migrated into the lobe, Fig. 3*a*). The bud may remain

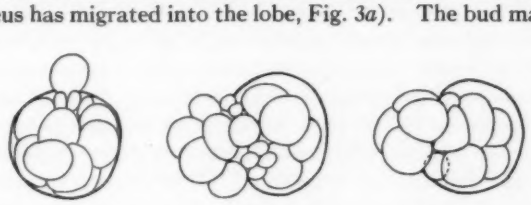


FIG. 3. *a*. Formation of small cells at the cut surface, because one of the earlier blastomeres has lost the greater part of its cytoplasm by a cytoplasmic protrusion. At the following divisions the descendants of the part left inside the membrane naturally were much smaller than the other blastomeres. *b*, *c*. Formation of small cells under the edge of the membrane, single (*c*) or in groups (*b*) at a distance from each other.

or fall off. This group of apparent micromeres on the cut side are thus formed by a process essentially different from that of the normal formation of micromeres. Occasionally the first cleavages in fragments may produce blastomeres of more or less unequal size with or without the formation of a bud. In that way, after several divisions, the smallest blastomere may give rise to a quartet of small cells, without any particular relation to the egg-axis, or to the cut side. The small cells of probably non-micromere origin may be of any number from one to five or six, or even more. It seems to me, moreover, that the pressure of the edges of the membrane on the base of large buds produces small cells, not only as descendants from a small half- or quarter-blastomere, but as a result of the pressure at the cleavage leading to

the 8- or 16-cell stage. This appears more probable in that several times small cells were found just under the edge of the membrane, and not always as a single group, but separated from each other (Fig. 3, *b*, *c*).

Thus there are several possibilities of obtaining small cells, often of a lighter color than the other blastomeres, and these small cells are not real micromeres. One would expect from this that small cells, micromeres and atypical small cells, would occur much more frequently in fragments than normally. But the contrary is the case. As mentioned above (pp. 300 and 301), micromere formation is very sensitive to mechanical injury; e.g. after shaking, an egg may show an equal cleavage, but the differentiation will not be influenced. In *Arbacia* fragments the micromere-formation seems to be inhibited very often.

As a result of these experiences, we have to deal with the following possibilities when dividing the unfertilized egg at random, if we assume the same arrangement of micromere material as in *Paracentrotus*. Vegetative and meridional halves ought to give whole cleavage, animal halves equal. But any fragment may cleave equally, i.e. if the micromere formation has become inhibited. Animal halves, and the other types of halves with inhibited micromere formation may show a more or less irregular pattern, the cells varying slightly in size. Any kind of fragment may form small cells which are not micromeres, either from the bud itself, close to the bud, or at any other place. It is naturally not easy to draw any conclusions from experiments where there are so many possibilities! According to Taylor, Tennent and Whitaker in *Lytechinus* all micromeres should appear on the cut side. According to Harnly, only the diploid halves form micromeres.

One diploid and 3 haploid fragments showed whole cleavage, with a bud opposite the micromeres. They thus behaved like the vegetative halves (p. 300 and Fig. 1*d*). Two diploid and 2 haploid fragments cleaved as whole eggs, having a lobe more or less on the side: they correspond to more or less meridional halves. Six diploid and 4 haploid fragments formed typical macro- and micromeres, as far as I could judge, but without any bud that made possible an orientation. Nine diploid and 5 haploid fragments cleaved equally, with or without bud. In 6 diploid and 4 haploid fragments the size of the blastomeres varied more or less, but no cells were as small as micromeres. In 7 diploid and 10 haploid fragments I found one or several small cells, the nature of which is open to question. They were in most cases formed either from or near the lobe, but sometimes not close to the lobe, or at a distance from each other.

These observations are at variance with those of Taylor, Tennent

and Whitaker on *Lytechinus*, as micromeres were sometimes formed at a distance from or even opposite the cut side, and they are not in agreement with the results of Harnly on *Arbacia*, as of the cases regarded as typical cleavage 9 were diploid while the same number, 9, were haploid.

Let us now examine the relations between cleavage patterns and differentiation. We speak below of three kinds of larvæ (Fig. 2). One corresponds to the animal halves of *Paracentrotus* and of *Arbacia* as we have seen in the preceding chapter, a blastula with enlarged apical tuft. One constitutes a gastrula with a large archenteron and with skeleton, derived from vegetative, meridional, and oblique vegetative-meridional halves. It is not essential to mention whether the larva later becomes a typical pluteus or only an ovoid larva. The important point is whether it gastrulates or not. The third type is a gastrula with a too small archenteron, such as is derived from oblique animal-meridional halves (Fig. 2, *e*, *f*).

Of the 18 fragments above characterized as showing whole cleavage, 17 developed to gastrulæ with large archentera and skeletons; one gave a blastula with enlarged apical tuft. The 24 fragments with equal (14) or slightly unequal (10) cleavages differentiated into 6 blastulæ, 2 gastrulæ with small, and 16 with large archentera. This result is what would be expected, as I consider that these two cleavage types correspond to animal halves and other types of half with suppressed micromeres. The 17 dubious cases must be described more in detail. In one pair one fragment showed two, the other one small cell inside the membrane, near the bud, but not quite in contact with it. Both developed into gastrulæ. I am inclined to interpret those as real micromeres, the halves as meridional fragments. In another case, some small light cells were formed at some distance from the bud, about 45°. The fragment differentiated into a gastrula. The nature of the cells is unknown. In one case the lobe itself divided into two small cells and one large cell. The former are certainly not micromeres, as the latter lay between them. The development led to a blastula with enlarged apical tuft. Three fragments had three or four small cells lying inside the membrane, just under the bud (Fig. 3*a*). I assume that these cells were so small because the expulsion of the bud deprived one of the quarter-blastomeres of a large part of its cytoplasm. Another reason for believing that these small cells are not micromeres is that the other blastomeres cannot be classified as meso- and macromeres. All three fragments gastrulated, but one of them had a slightly enlarged apical tuft and only a small archenteron. In four cases a large part of the fragment had protruded through the

opening in the fertilization membrane and divided, just as well as the part within the membrane. In all four fragments small cells were formed, and in all they were situated under the edge of the membrane (Fig. *3b, c*). I do not believe that these small cells are comparable to micromeres, as in three of the four fragments they were found single or in groups at a distance from each other (Fig. *3b, c*). One developed into a gastrula, two into blastulæ of the animal type, and one died. The remaining six fragments had one or several small cells, but the sizes of the other cells were so varied, and the position of the "micromeres" sometimes so scattered, that we cannot speak of a regular cleavage pattern with micromeres. In one case a close examination showed that the two apparent micromeres were not cells at all, only two small buds without nuclei. Three of these six fragments gastrulated, two developed into blastulæ with enlarged apical tufts, and one died.

C. Conclusions

In this experiment we divided the egg with a random section in relation to the egg-axis, the plane of section being oriented in relation to the egg-nucleus (Fig. 2). The entoderm- and the skeleton-forming material cannot be limited to the region between the egg nucleus and the center of the egg as both the diploid and the haploid larvæ form archentera and skeletons with about equal frequency (in 27 and 26 cases respectively, from the pairs) and as the blastulæ with enlarged apical tufts were derived not only from haploid (6 cases) but also from diploid fragments (5 cases). Nor can the micromere-forming material be localized between the egg-nucleus and the center of the egg, as Harnly stated. Of the 18 cleavage stages that were considered to possess typical micromeres, 9 were diploid and just as many—9—haploid. Fifteen diploid and 9 haploid fragments formed no micromeres, showing equal or slightly irregular cleavage. In 7 diploid and 10 haploid fragments, small cells were found, the nature of which has been discussed in detail.

As 5 diploid and 6 haploid fragments of the pairs and 1 diploid and 1 haploid of the single halves failed to gastrulate, the entoderm- and skeleton-forming material cannot have the same widespread distribution in *Arbacia* as in *Lytechinus*, in which, according to Tennent, Taylor and Whitaker, it occupies 19/20 of the volume of the egg. In that case every healthy fragment of the size of a half-egg would have gastrulated. Furthermore, the micromeres were not always formed on the cut side. In 4 cases they were found antipolar to the cytoplasmic bud, in 4 this bud was situated laterally in relation to the

cleavage axis. In 24 fragments no micromeres were formed. In some of the 17 dubious cases we could show that the small cells on the cut side were not real micromeres.

On the contrary, our results with one exception are in conformity with the view that both the micromere-forming and the entoderm- and skeleton-forming material is localized in a part of the *Arbacia* egg that is smaller than half the volume and comparable to the vegetative quarter of the *Paracentrotus* egg. Both diploid and haploid halves could gastrulate or differentiate into blastulæ of the animal type. Both diploid and haploid halves could form micromeres. These could appear on the cut side, opposite the cut side, or on any other side. With one exception, all fragments that formed "real" micromeres gastrulated. Fragments with equal or slightly irregular cleavage gave all kinds of larvæ, in accordance with the view that they comprise the animal halves and any other kind of halves with suppressed micromeres. Of the dubious cases, some with small cells developed into blastulæ with enlarged apical tuft, i.e. as animal halves; but it was shown above that in all probability those small cells were not real micromeres, as either the whole cleavage stage was very irregular, or the small cells were located at some distance from each other. The only case that does not fit in with the assumption of a localization of both the micromere-forming and the entoderm- and skeleton-forming material in the vegetative part of the egg was one fragment which was supposed to have a typical 16-cell stage but differentiated into a blastula with large apical tuft (p. 305). Was that a case of micromere formation of an animal half, or was the interpretation of the cleavage stage wrong?

IV. Discussion

Although the micromere- and the skeleton-forming material occupy the same region in the *Paracentrotus* egg,—roughly speaking, the most vegetative quarter—the factors causing the formation of the micromeres and those causing the formation of the primary mesenchyme of the skeleton-forming cells are not identical. As many authors have pointed out, normal primary mesenchyme is formed even if the micromeres are suppressed.

Harnly (1926) studied only the localization of the micromere material, but we have also paid attention to the differentiation of the fragments which were isolated in order to test his results. The outcome of his experiments, that the micromere-forming material in *Arbacia* prior to fertilization lies between the nucleus and the center of the egg, is contradicted by the two series of experiments described above. Animal halves did not form any micromeres, whereas the

vegetative fragments did, and in both cases irrespective of the position of the egg nucleus: the animal and the vegetative fragments could be diploid or haploid; the egg nucleus could at the operation lie close to the plane of section or at some distance from it. When the egg was divided into two equal halves by a section as far from the nucleus as possible, Harnly (see his Table 3 and Fig. 1*a*) obtained normal cleavage only in the nucleated fragments (23), whereas 28 haploid fragments showed "two tiers of eight equal cells." The pairs described above formed (in the fairly clear cases) micromeres in 9 diploid and 9 haploid, and no micromeres in 15 diploid and 9 haploid fragments.

In the light of my experiences, I have difficulty in understanding Harnly's results. He found in his experiment (Table 1) that "in no case did a nucleated half that divided normally through the first three cleavages give other than a normal fourth cleavage." Of 132 diploid fragments 30 showed normal fourth cleavage, 38 irregular and partial first cleavage, 50 endoplasmic buds (not studied), and 9 were undetermined. In Tables 3–5 no buds and no irregular and partial first cleavage are recorded. When the eggs were fragmented, as in our Fig. 2*a*, practically all diploid fragments segmented normally, but the haploid formed "two tiers of eight equal cells." When the eggs were divided into two equal halves by a section close to the nucleus, all the fragments that were determined, diploid or haploid, segmented in a normal way, except a few haploid ones, which had only two or three macro- and micromeres. As we have seen, I have not found that fundamental difference between the diploid and haploid fragments cut as in Fig. 2*a*. Both kinds could form micromeres, and both kinds could show equal or slightly irregular cleavage. When equal, the blastomeres were hardly ever arranged as regularly as in two tiers of eight cells, as Harnly states for his 28 enucleated halves. Harnly also isolated non-nucleated fragments containing two-thirds or more of the material of the egg. One fragment was undetermined, 18 segmented normally. Harnly's explanation is that these large haploid fragments contained all the micromere-forming material lying between the nucleus and the center of the egg. A more or less typical cleavage would in this case be expected also when assuming a localization of the micromere material in the vegetative quarter of the egg, since only very few of the fragments would be completely devoid of such material.

Harnly (1926) also divided fertilized eggs into two equal halves. According to Harnly, the fusion nucleus is to be found in the egg-axis, just above the equator; Harnly tried to cut equatorially. The fertilization membrane could not be divided, the two halves remained inside the membrane, flattened against each other. Only the half

containing the egg nucleus segmented. Three types of cleavage were found: whole cleavage with the micromeres opposite the cut side, whole cleavage with the micromeres close to the cut side, and equal cleavage. Harnly concludes from his experiments that, within five minutes after fertilization, the micromere-forming material changes its position. Both the nucleus and the micromere material are now oriented with regard to the primary axis. Harnly does not expressly state where along the axis the micromere material is now located, but he seems to indicate a central position. As far as I can see, his results accord just as well with the view that the micromere material is restricted to the vegetative part of the egg. The equal cleavage (the largest number, as the nucleus in most cases was lying above the equator) belongs to the animal halves; the whole cleavage with the micromeres opposite the cut side corresponds to the cases in which the nucleus came to lie in the vegetative half; and the two fragments with the micromeres close to the plane of division are probably, as Harnly himself presumes, meridional halves.

Tennent, Taylor and Whitaker (1929, p. 66) state that their results "have compelled the conclusion that in the unfertilized egg of *Lytechinus* there is an animal polar cap and a superficial layer of ectoderm-forming material surrounding a core of undifferentiated material that is potential endoderm, primary and secondary mesenchyme, and mesoderm." The presence of an animal cap of ectoderm-forming material, occupying 1/20 of the volume of the egg (*loc. cit.*, p. 66) was concluded from the fact that gastrulæ were obtained from vegetative fragments of 1/11 the volume of the egg (but there is only one of that size, No. 1926 253), while none of the larvæ from fragments of equal size from the region of the animal pole developed beyond the stage of blastulæ with mesenchyme (No. 1926 276, 279; see also pp. 47, 63, 64, 66). The smallest fragment that developed into a blastula was 1/21 of the volume of the egg. These results point, as it seems to me, rather to the size 1/11 than 1/20 for the animal polar cap. The reason for the conclusion that there is a superficial layer of presumptive ectoderm also in the other parts of the egg is not given in the paper in question, as far as I can find. But in the preliminary report by Taylor, Tennent and Whitaker (1926), the same conclusion has been drawn from the fact that small superficial fragments—with a diameter of one-fifth of that of the egg—only gave blastulæ with or without mesenchyme, while larger, but still small fragments gastrulated. A fragment with a diameter of one-fifth of that of the egg has a volume that is only 1/125 of that of the egg. Nobody has so far been able to rear gastrulæ from such small fragments. The smallest blastomere that has been

observed to gastrulate is a half-macromere (Hörstadius, 1936*a*). Its volume is larger than 1/32 but smaller than 1/16 of the egg. Morgan (1895) and Driesch (1900, 1902) estimated the smallest egg fragment that could gastrulate at 1/40–1/60 and 1/32 respectively of the volume of the egg. But these results are very uncertain, as the size of the fragment was concluded from the size of the larvæ. In the full report, Tennent, Taylor and Whitaker (1929, p. 62) state, contrary to the preliminary note, that the smallest fragment that gave a gastrula had 1/11 the volume of the egg, the smallest that gave a blastula 1/21 (*cf.* above). Under such circumstances I cannot see how the idea of a superficial layer of ectoderm-forming material round the whole egg can be supported. Very small fragments probably do not gastrulate because they are too small, not because of lack of presumptive archenteron (if derived from a vegetative part of the egg). As the smallest blastulæ obtained were derived from fragments measuring 1/21 of the volume of the egg, no blastulæ seem to exist that could prove the presence of a superficial layer of ectoderm-forming material outside the entoderm-mesenchyme-forming substance that, in *Lytechinus*, is supposed to occupy 19/20 (10/11?) of the egg.

We now turn to a comparison of the facts leading to the idea of an animal cap of ectoderm material, occupying only 1/20 (1/11?) of the egg in *Lytechinus*, with the facts demonstrating that in *Paracentrotus* and *Arbacia* the presumptive ectoderm is distributed over more than half the egg. Animal halves of the unfertilized egg of *Paracentrotus*, oriented at the operation by means of the pigment ring, never gastrulated—*cf.* p. 296. None of our 12 animal halves of *Arbacia* (orientation by means of the micropyle) gastrulated, and all showed an enlarged apical tuft. Of the halves cut at random, many differentiated in the same way (Fig. 2). Tennent, Taylor and Whitaker (1929, p. 57) report the history of 27 pairs of fragments isolated by horizontal sections. But of these pairs, both fragments reached gastrulation age only in 9 cases. If the presumptive entoderm- and mesenchyme-forming material occupies 19/20 (10/11?) of the egg, every horizontal fragment larger than 1/20 (1/11?) should gastrulate. This was the case in 6 pairs, the relative size of the fragments varying from 1 : 1 to 1 : 3.4 (No. 1924 8, 42, 44, 54, 61, 1926 230). In one case both gastrulated with the size difference 10 : 1 (No. 1926 253), the animal fragment being the larger. In two pairs, however, the animal partner did not gastrulate. In the one pair its volume was only 1/17 of that of the egg (No. 1926 229), but in the other the fragments were of approximately the same size (1 : 13, No. 1926 231). The animal fragment formed a blastula without mesenchyme. In addition, 3 of the single larvæ, all of half

size (No. 1924, 5, 45, 79) remained as blastulæ, while 11 gastrulated. These results, with 4 fragments of approximately half size differentiating only to blastulæ, are at variance with the assumption that the presumptive entoderm and mesenchyme occupies 19/20 or even 10/11 of the egg.

Of 21 vertical sections in *Lytechinus* both partners developed to gastrulation age only in 5 pairs. Here we should expect gastrulation in every fragment of sufficient age (10 hours), and it occurred in 4 pairs (No. 1924 40, 53, 56, 80). But in the fifth pair (No. 1924 60) one fragment only formed a "blastula with mesenchyme," while the other developed to a pluteus. These two fragments were of equal size. Furthermore, not less than 3 (No. 1924 23, 25, 1926 267) of the other 10 vertical halves (only one partner living) that lived longer than 10 hours failed to gastrulate.

Seven pairs of fragments obtained by diagonal sections (Tennent, Taylor and Whitaker, 1929, p. 58) were reared (No. 1926 275, 279–283, 285). In four cases the small, oblique animal fragment, of size 1/6, 1/11, 1/11, 1/21, formed a blastula with mesenchyme, while the larger vegetative fragment gastrulated. In two cases the animal fragment was the larger (5 : 1, 4 : 1); both partners gastrulated. In the seventh pair also both developed to gastrulæ, the size of the oblique animal fragment being 1/7 of that of the vegetative.

The blastulæ obtained from the animal fragments are said to have a thickened posterior wall and often a number of mesenchyme cells. No mention is made of the size of the apical tuft in these blastulæ. Nevertheless, I get the impression from the drawings of such blastulæ (Figs. 44, 47) that they are typical animal fragments. Thus the thickened wall would correspond, not to the posterior, but to the animal and ventral side (*cf.* Lindahl, 1933; Hörstadius, 1935, p. 286, 1936*a*, p. 56). This interpretation is supported by the fact that the "mesenchyme" cells in the figures are located further towards the thin wall than towards the thickened wall. Furthermore, I very much doubt whether all the cells reported as mesenchyme cells have that character. In fragments, very often some cells pathologically migrate into the blastocœl. Many isolated animal halves of *Paracentrotus* may have a few, or a large number of cells free in the interior of the blastula. They have nothing to do with real mesenchyme cells. They may, of course, also appear in meridional and vegetative fragments, together with primary mesenchyme cells. It requires an intimate knowledge of the material to recognize the real mesenchyme cells from these pathological cells, and also to interpret correctly the animal blastulæ. The cells in the blastulæ (Figs. 44 and 47) are rather

varied in size. This indicates, it seems to me, that here we have not to deal with real primary mesenchyme. Furthermore, the assumption that the mesenchyme material occupies 19/20 of the egg is contradicted by the fact that Tennent, Taylor and Whitaker report several blastulæ of half size without mesenchyme (No. 1924 5, 23, 25, 1926 231).

The fact that 4 of the horizontal and 4 of the vertical fragments of half-size did not gastrulate is incompatible with the conception of the presumptive entoderm occupying 19/20 (10/11?) of the egg. It rather shows that it is restricted to less than the half of the egg. Also the small diagonal fragments illustrate the same thing, as in some cases they contain material from the equator region without gastrulating.

The fact that both partners did gastrulate in 7 of the 9 animal-vegetative pairs, and that 4 of 20 vertical fragments did not gastrulate, seems to indicate that the orientation was not always that desired. When discussing the localization of the entoderm and mesenchyme material in *Arbacia* with some colleagues at Woods Hole in 1936, I said that one ought to obtain blastulæ of animal type from, roughly speaking, 15 per cent of the halves isolated at random, if the same conditions as in *Paracentrotus* prevailed. My material of random sections on *Arbacia* numbers 14 blastulæ with enlarged apical tuft and 65 larvæ that gastrulated. Thus 18 per cent did not gastrulate. This indicates that the presumptive entoderm occupies less than half of the egg, otherwise the animal blastulæ would have been much fewer. Eight of the *Lytechinus* fragments of approximately half-size remained in the blastula stage, while 40 developed into gastrulæ or plutei. Thus 17 per cent did not gastrulate. These figures of all the horizontal and vertical sections together raise some doubts as to the accuracy of the orientation of the cut, particularly as 4 of 20 meridional fragments only formed blastulæ. Has the plane of section often been different from that desired? One possibility is that the egg slipped or turned during the operation. The three authors state (1929, p. 6) that the operator could control this. The micropyle and the polar bodies served as landmarks. Are they reliable? Tennent, Taylor and Whitaker (1929, p. 12) regarded it as possible that the egg might rotate in its jelly and in that manner make the micropyle of uncertain value as a landmark. "No evidence of rotation was found. Careful examination showed the polar bodies lying at the base of the micropyle. In one instance only were they out of line as much as five degrees." But in the preliminary report (1926) it is stated that "our preliminary observations having convinced us that the egg might be rotated in its jelly, we felt that our orientation of the egg was dependable only when the polar bodies could be found at the base of the micropyle." But

is it not possible that the egg can rotate inside the jelly, the polar bodies being held by the micropyle? (Hörstadius, 1928, p. 91.) If so, the egg-axis may lie at any angle to the axis indicated by the micropyle and the polar bodies. Or has the whole egg rotated during the slow sectioning with the micromanipulator?

From the fact that, in certain fragments obtained by vertical section, the first cleavage amphiaster lies temporarily in the short axis of the cell, and that in those cases the size of the cleavage cells is atypical, Tennent, Taylor and Whitaker (1929, pp. 41–48) conclude that there is a prelocalization of an entoderm-forming substance. I can but support the reservation of Dr. Taylor (*loc. cit.*, p. 47), who doubts the significance of cell-size as indicative of a segregation of entoderm-forming material in the unfertilized egg. In *Paracentrotus*, under certain circumstances, the four animal cells of the 8-cell stage may be much larger than the four vegetative (vorzeitige Mikromeren), or the spindles of the second and third division may stand obliquely, or the spindle of the first division in an animal-vegetative direction instead of equatorial, or a vegetative half may show a typical whole cleavage, all without any change of the position or amount of not only the micromere-, but also the entoderm-forming material (see Hörstadius, 1928, pp. 10, 14). As far as I can see, the results in question only permit conclusions as to the cleavage factors, not as to the localization of presumptive entoderm.

Both Harnly (1926) and Tennent, Taylor and Whitaker (1929) state that the first two furrows in fragments stand at right angles to the surface of section, irrespective of the orientation of the egg. The last-mentioned authors maintain that a new polar axis has been established (*loc. cit.*, p. 66). But, as we have seen above, the position of the first two furrows may be entirely independent of the polar axis of the egg and larva. In order to determine whether a new polar axis has been established, one has to ascertain by local vital staining whether the cleavage axis coincides with the axis apical organ—blastopore in the gastrula. In *Paracentrotus* the micromeres are always formed at the vegetative pole, irrespective of the position of the first two furrows, and differentiation takes place in accordance with the original egg-axis (Hörstadius, 1928, p. 10; 1936*a*, p. 53). Also, if micromeres are formed on the cut surface at the intersection of the first two cleavage planes, as stated for *Lytechinus*, this does not necessarily mean a change of polarity, as the factors for micromere formation and skeleton and entoderm formation are not identical (*cf.* the paragraph above and p. 307).

The statement that the micromeres are always formed on the cut

side in *Lytechinus* is surprising. Tennent, Taylor and Whitaker have studied the cleavage of fragments in a great many cases, and the drawings seem convincing, although they look rather schematized. But the statements are perhaps not entirely conclusive, as the micromeres in our vegetative *Arbacia* fragments were formed opposite the cut side and as the three authors (1929, pp. 36–39) also made some experiments on *Arbacia* at Woods Hole, which do not agree with those presented here. Only 14 of 45 operations were either fully or partially successful. If we count all the fragments that developed, single and pairs, 1–4 micromeres were found in 6 vegetative and 6 animal fragments, while 2 vegetative and 1 animal fragment cleaved irregularly, and 5 animal and 2 vegetative divided equally. Nothing is said as to the position of the micromeres in relation to the cut surface. These results contrast markedly with mine: 11 of 12 vegetative fragments showed micromeres, all opposite the cut side, while the animal halves did not form any real micromeres. Tennent, Taylor and Whitaker do not discuss any of the possible sources of error regarding the micromere formation. Moreover, perhaps the free hand is a better instrument for cutting the eggs than the micromanipulator, which may work so slowly that a rotation of the egg after the final orientation is possible. A renewed investigation on *Lytechinus*, as well as on *Arbacia*, with attention payed particularly to the orientation of the cut and the possible formation of false micromeres, with a large number of larvæ that reach full differentiation, and with local vital staining to study the relation of the axis of the egg to that of the fragment and the relation of the micromeres to the cut surface, would be very desirable.

The critical discussion may be *summarized* as follows. Harnly's assumption (1926) that the micromere-forming material in the unfertilized egg of *Arbacia* is situated between the nucleus and the center of the egg does not hold, as vegetative halves formed micromeres, and animal halves showed no micromeres, in both cases irrespective of the position of the nucleus, and as nucleated and non-nucleated halves isolated by a cut perpendicular to the line nucleus-center of the egg (Fig. 2*a*) formed micromeres with equal frequency. The conclusions of Tennent, Taylor and Whitaker (1929) that the egg of *Lytechinus* has an animal polar cap occupying only 1/20 (1/11?) of the volume of the egg, and a superficial layer of ectoderm-forming material are contradicted by their own results. The conclusion is drawn from the differentiation of one animal and two vegetative fragments of the size 1/11. But there are diagonal fragments of the same size, or larger, reaching down to the equator, that do not gastrulate. Moreover, several animal and meridional fragments of half size and sufficient

age did not gastrulate. These facts indicate that the presumptive ectoderm occupies at least half the egg. The fact that several meridional halves also differentiated as an animal fragment arouses the suspicion that the plane of section has not always been that desired, which would explain many of the discrepancies between *Lytechinus* and *Paracentrotus*. It is questionable whether some of the cells described as mesenchyme cells are not pathological. The position of the first two cleavage planes does not necessarily indicate the position of the axis of the larva. In fragments of *Lytechinus* eggs the micromeres are reported by Tennent, Taylor and Whitaker to be formed on the cut side. In *Arbacia* I found the micromeres formed at the vegetative pole, irrespective of the position of the section. The fact that the results of Tennent, Taylor and Whitaker as to the localization of the micromere-forming material in *Arbacia* do not agree with mine, which in turn confirm those on *Paracentrotus*, makes a reinvestigation of *Lytechinus* also desirable.

V. Summary

Animal halves of unfertilized eggs of *Arbacia punctulata* segmented equally and developed into larvæ of animal type: blastulæ with enlarged apical tuft. Vegetative halves formed micromeres antipolar to the cut side, and gastrulated, differentiating into ovoid larvæ or plutei. Meridional halves all gastrulated. The type of development was independent of the presence or absence of the egg nucleus (Fig. 1).

Unfertilized eggs were divided into approximately equal halves by a section perpendicular to the line nucleus-center of the egg (Fig. 2*a*), the plane of section thus being laid as far away from the nucleus as possible, and at random in relation to the egg-axis. Nucleated and non-nucleated fragments formed micromeres and gastrulated with equal frequency. Some of the fragments differentiated as animal halves (Fig. 2). Atypical small cells, particularly on the cut side, which may be mistaken for micromeres are often formed.

The results indicate that the micromere-forming and the entoderm- and the skeleton-forming material in *Arbacia* is located in the most vegetative part of the unfertilized egg, occupying less than half the volume of the egg. The results of Harnly (1926) on *Arbacia* and of Tennent, Taylor and Whitaker (1929) on *Lytechinus* are critically discussed.

Literature Cited

Boveri, Theodor, 1901*a*. Die Polarität von Ovocyte, Ei und Larve des Strongylocentrotus lividus. *Zool. Jahrb., Abt. Anat. u. Ont.*, **14**: 630.

Boveri, Theodor, 1901*b*. Über die Polarität des Seeigel-Eies. *Verhandl. d. phys.-med. Gesellschaft. Würzburg*, **34**: 145.

BOVERI, THEODOR, 1902. Über mehrpolige Mitosen als Mittel zur Analyse des Zellkerns. *Verhand. d. phys.-med. Gesellschaft Würzburg*, **35**: 67.

DRIESCH, HANS, 1896. Betrachtungen über die Organisation des Eies und ihre Genese. *Arch. Entw.-mech.*, **4**: 75.

DRIESCH, HANS, 1898. Über rein mütterliche Charaktere an Bastardlarven von Echiniden. *Arch. Entw.-mech.*, **7**: 65.

DRIESCH, HANS, 1899. Die Lokalisation morphogenetischer Vorgänge. *Arch Entw.-mech.*, **8**: 35.

DRIESCH, HANS, 1900. Die isolirten Blastomeren des Echinidenkeimes. *Arch. Entw.-mech.*, **10**: 361.

DRIESCH, HANS, 1902. Neue Ergänzungen zur Entwickelungsphysiologie des Echinidenkeimes. *Arch. Entw.-mech.*, **14**: 500.

HARNLY, MORRIS H., 1926. Localization of the micromere material in the cytoplasm of the egg of Arbacia. *Jour. Exper. Zoöl.*, **45**: 319.

HOADLEY, LEIGH, 1934. The relation between the position of the female pronucleus and the polar bodies in the unfertilized egg of Arbacia punctulata. *Biol. Bull.*, **67**: 220.

HÖRSTADIUS, SVEN, 1928. Über die Determination des Keimes bei Echinodermen. *Acta Zoologica, Stockholm*, **9**: 1.

HÖRSTADIUS, SVEN, 1935. Über die Determination im Verlaufe der Eiachse bei Seeigeln. *Pubbl. Staz. Zool., Naples*, **14**: 251.

HÖRSTADIUS, SVEN, 1936*a*. Weitere Studien über die Determination im Verlaufe der Eiachse bei Seeigeln. *W. Roux. Arch. Entw.-mech.*, **135**: 1.

HÖRSTADIUS, SVEN, 1936*b*. Studien über heterosperme Seeigelmerogone nebst Bemerkungen über einige Keimblattchimären. *Mém. Mus. d'Hist. Nat. de Belgique*, Ser. 2, Fasc. **3**: 801.

LINDAHL, P. E., 1933. Über "animalisierte" und "vegetativisierte" Seeigellarven. *W. Roux. Arch. Entw.-mech.*, **128**: 661.

MORGAN, THOMAS HUNT, 1894. Experimental studies on Echinoderm eggs. *Anat. Anzeig.*, **9**: 141.

MORGAN, THOMAS HUNT, 1895. Studies of the "partial" larvæ of Sphærechinus. *Arch. Entw.-mech.*, **2**: 81.

TAYLOR, C. V., AND D. H. TENNENT, 1924. Preliminary report on the development of egg fragments. *Carnegie Inst. Wash. Yearbook* No. 23.

TAYLOR, C. V., D. H. TENNENT AND D. M. WHITAKER, 1926. Investigation on organization of Echinoderm egg. *Carnegie Inst. Yearbook* 25.

TENNENT, D. H., C. V. TAYLOR AND D. M. WHITAKER, 1929. An investigation on organization in a sea-urchin egg. *Carnegie Inst. Wash. Publ.* No. 391.

TERNI, T., 1914. Studio sulle larve atipiche (blastule permanenti) degli Echinoidi. *Mitt. Zool. Stat. Neapel*, **22**: 59.

VON UBISCH, L., 1925. Entwicklungsphysiologische Studien an Seeigelkeimen. *Zeitschr. wiss. Zool.*, **124**: 361.

ZOJA, R., 1895. Sullo sviluppo dei blastomeri isolati dalle uova di alcune meduse (e di altri organismi). *Arch. Entw.-mech.*, **2**: 1.

EXPERIMENTS ON DETERMINATION IN THE EARLY DEVELOPMENT OF CEREBRATULUS LACTEUS

SVEN HÖRSTADIUS

(From the Zoötomical Institute, University of Stockholm, and the Marine Biological Laboratory, Woods Hole, Massachusetts)

I. Introduction

The eggs of nemerteans, annelids, gastropods, lamellibranchs and scaphopods show the spiral type of cleavage. Most of these eggs,—those of the annelids and mollusks,—are assigned to the mosaic type of determination. Their isolated blastomeres have been found to develop in accordance with their prospective significance. None of these eggs has, however, been investigated in such detail as the sea urchin egg, of which we know that some parts may develop into a complete larva (Driesch, 1891, etc.) and that other parts may differentiate less typically than in the case of normal development, owing to the fact that interactions have to take place between different parts of the egg, bringing the formation of some organs (Hörstadius, 1928, 1935, 1936). [von Ubisch (1936) has denied the rôle of interactions in the sea urchin egg. However, new isolation and implantation experiments, as well as constriction experiments (unpublished), completely confirm my earlier results as to the interactions.] The scope of this investigation was to see whether parts of an egg of the mosaic type might not show traces of interaction when transplanted atypically upon each other. As I could not find any annelid or mollusk egg so suitable for isolations and transplantations as the *Cerebratulus* egg, I had recourse to the latter. This egg shows a spiral cleavage but is not wholly a mosaic egg. It belongs to the regulation type insofar as fragments of the uncleaved egg and isolated half- and perhaps quarter-blastomeres may give rise to harmonic dwarf larvæ. But, on the other hand, the investigations of E. B. Wilson (1903), Zeleny (1904) and Yatsu (1910) indicate that the animal and vegetative parts of cleavage stages differentiate as fragments. Thus in that respect we seem to have to deal with a mosaic.

The cell-lineage of the nemertean egg has never been worked out. For that reason, in order to be able to interpret rightly the results of the isolation and transplantation experiments, we had first to investigate the prospective significance of the cell layers. As the experiments of the authors mentioned above are rather incomplete, we also have had to repeat and extend the study of isolated fragments. The results of previous investigators will be mentioned below in connection with each corresponding experiment.

II. Material and Methods

The investigation was undertaken in July and the first part of August, 1936, at Woods Hole. The worms (*Cerebratulus lacteus* Verrill) were sent in several deliveries in ice-cooled jars from Lynn, north of Boston, and kept in an ice-box. In order to obtain the eggs, a piece of a female was put into a piece of cheesecloth in sea water. The cheesecloth retains the slime but allows the eggs to fall through. Coe (1899) has shown that the germinal vesicle gradually fades away, when the eggs come out in the sea water and the first polar spindle is formed, but the spindle remains in the metaphase until fertilization occurs. In order that uniform conditions as regards maturation and fertilization might be secured, the sperms were added half an hour after the shedding of the eggs (Zeleny, 1904). The egg forms no fertilization membrane but is surrounded by a jelly and a soft membrane, which are easily removed by gentle shaking.

The blastomeres of the 2-, 4-, and 8-cell stages are easily separated with glass needles. The separation of the four layers of the 16-cell stage is more difficult, as the blastomeres from two adjacent layers lie partly between each other (Fig. 1, *B*). But it can be done by

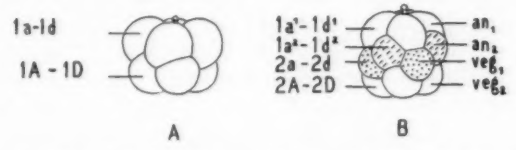


Fig. 1. Eight-cell stage (*A*) and 16-cell stage (*B*) of *Cerebratulus*.

cutting before the fourth cell division is quite completed, because at that moment the cells have not yet sunk in between each other.

For transplantation the fragments were placed one on top of the other in a cavity of appropriate size, made in a celluloid plate, and a small glass sphere placed on top of the upper fragment to produce pressure (Hörstadius, 1928). In some cases the fragments fused after being made to adhere by a brief, gentle pressure of the needle. Local staining (of a single blastomere or of a layer of blastomeres) was made by leaning the cell or cells in question against a piece of agar (Vogt, 1923, 1925; v. Ubisch, 1925) or by staining an isolated cell layer and then transplanting it (Hörstadius, 1928, 1935).

The number of experiments is rather limited. This is due partly to the fact that the termination of the breeding season already in early August cut short my experiments before the completion of an adequate study, partly to the fact that in many series nearly all larvæ died.

The reason for this was not a pathological condition of the larvæ. They looked very healthy until they suddenly disappeared by getting caught and expanded, just as if exploded, by the surface tension. The death ratio was considerably reduced when the larvæ were reared in a large drop of water between a slide and a coverslip on plasticine feet and protected from evaporation by vaseline.

III. Normal Development. Nomenclature

The following facts regarding the normal development have to be borne in mind. The *Cerebratulus* egg is very dark brown, opaque. But the polarity of the just-laid egg may be accurately determined partly by a conical protuberance at the vegetative pole, partly by a clear area (the fading germinal vesicle) at the animal pole. Later the polar bodies at the animal pole give a good landmark. They are also present in the cleavage stages.

The first two furrows are meridional, the blastomeres of the 4-cell stage all being of the same size. Thus we have no predominance of the D-quadrant in the nemertean egg. The third cleavage is dexiotropic, forming four micromeres (1a–1d) and four macromeres (1A–1D), but the micromeres are larger than the macromeres, as Coe (1899) first described (Fig. 1, *A*). At the next (leiotropic) cleavage, the micromeres (the first quartet of micromeres, 1a–1d) are divided into two layers each of four cells ($1a^1$–$1d^1$ and $1a^2$–$1d^2$), and the macromeres bud off a second quartet of micromeres (2a–2d). The macromeres are now called 2A–2D (Fig. 1, *B*).

The use of the words micro- and macromeres at the 8-cell stage is rather confusing, as the micro- are larger than the macromeres. In the following account we prefer to call them the animal and the vegetative cells of the 8-cell stage. Instead of speaking of the two layers derived from the first quartet of micromeres ($1a^1$–$1d^1$, $1a^2$–$1d^2$), of the second quartet of micromeres (2a–2d), and of the macromeres (2A–2D) of the 16-cell stage, we may simply designate the four layers as an_1, an_2, veg_1, and veg_2 (Fig. 1, *B*), as has been done with the four layers in the sea urchin egg (Hörstadius, 1931, 1935). The composition of a larva may then be expressed by a formula, e.g., $an_1 + an_2 + veg_1$ means a larva from which veg_2 has been removed, etc.

The gastrula is still rather opaque, but the fully differentiated larva, the pilidium, is quite transparent. The blastopore forms the mouth, leading into an œsophagus and stomach; there is no intestine or anus (Fig. 2, *A*). One or two days after gastrulation two lappets grow out on the left and right sides of the mouth. The whole surface of the pilidium is ciliated, but the lappets are bordered by a special

ciliated band where the cilia are longer, more concentrated, and beat in a characteristic way as compared with the cilia of the rest of the ectoderm. The cells of the ciliated band are more yellowish than the other ectoderm cells. At the animal pole we find an apical organ which is a sense organ, a long flagellum in a thickened pit of the ectoderm. The flagellum is not a single structure but is composed of a bundle of fine threads.

The right and left sides of the pilidium evidently are those which bear the lappets where the lappets are formed. As there is no anus it is difficult to say what corresponds to the dorsal, what to the ventral side. In the gastrula we call that axis dorso-ventral which is perpendicular both to the animal-vegetative and the right-left axes, but in the pilidium the dorso-ventral axis probably, as the ventral side presumably is so short, only forms an acute angle with the egg (animal-vegetative) axis. After metamorphosis the egg axis corresponds to the dorso-ventral axis of the worm.

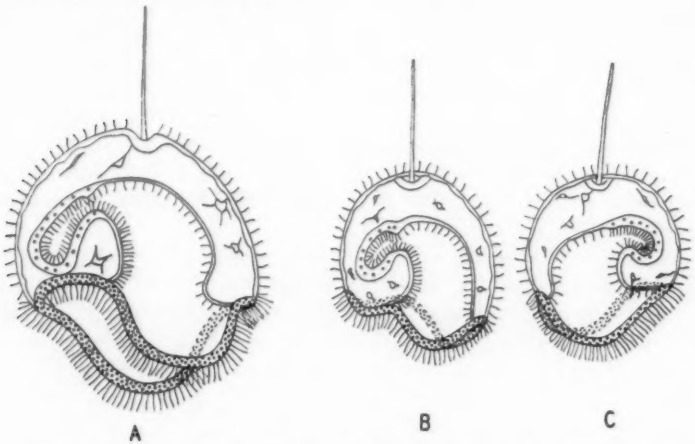


FIG. 2. *A*. Normal pilidium. *B, C*. Pilidia from the isolated two half-blastomeres of one egg.

IV. THE PROSPECTIVE SIGNIFICANCE OF an_1, an_2, veg_1, AND veg_2

The four animal cells of the 8-cell stage were stained by leaning them against a piece of agar. The whole ectoderm down to the ciliated band was blue, and there was also an abundance of blue granules in the band. When the four vegetative cells of the 8-cell stage were stained, the stomach, the œsophagus, the inner sides of the lappets, and the ciliated band were blue. Thus also in this case, we found the ciliated band markedly stained. When the animal cells

were stained, the upper, most animal part of the whole band (with one probable exception, see below) contained blue granules, and when the vegetative cells had been stained, the color was restricted more or less to the lower, vegetative part of the band. It is very difficult to trace the limit of the stained area with certainty in *Cerebratulus*, but I think it is evident that the ciliated band is composed of material both from the animal and the vegetative cells.

It was observed in several cases that on one day the young pilidium showed the ectoderm, including the ciliated band, bluish (staining of the animal cells); the next day the stomach too had turned blue. It was noted, however, that the œsophagus and the inner sides of the lappets were still unaffected by the Nile blue. Thus it would appear that we are not dealing with a general diffusion of the stain. It seems probable that at this stage the stomach acts as an excretory organ, as is the case with the digestive tract of turbellarians (Westblad, 1923).

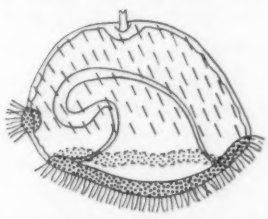


FIG. 3. In the 16-cell stage the four most animal cells, an_1, were isolated, vitally stained and transplanted back on the an_2 cells. The animal fragment was probably rotated 180° at the transplantation: note the little patch of ciliated band too high up on the posterior side. Normally it probably constitutes the most anterior part of the band. Stained area stippled.

Staining of the four veg_2-cells of the 16-cell stage resulted in a blue stomach. Whether a small part of the veg_2-material is or can be used to form a part of the œsophagus I cannot tell,—the limit of the stained area was not sufficiently sharp.

Also in staining the four an_1-cells I had great difficulties in observing the colored line. It seems to me that these four cells form the greater part of the pretrochal ectoderm down to about the equator of the pilidium. Thus an_2 would form the ectoderm on the outside of the lappets and a part of the ciliated band. I have the impression that the an_1-material in the anterior part of the larva goes down to the ciliated band and contributes to the formation of its foremost part, whereas posteriorly it does not reach so far down. This view is supported by the following result. For the purpose of obtaining a sharper limit of the stain, the four an_1 cells were cut off, stained, and replaced. In one case they probably happened to be rotated 180°, because the

larva showed a short piece of ciliated band way up from the ciliated band at the posterior end of the pilidium, and the stain border line ran from this isolated piece of band to the band at the anterior end of the larva (Fig. 3). This would indicate that the part of the ciliated band that is normally derived from the an_1-material at the front end of the larva has undergone self-differentiation too high up on the posterior side.

The prospective significance of the four layers thus seems to be as follows: an_1 gives the ectoderm of the upper half of the pilidium, including a very small piece of the ciliated band. an_2 forms the rest of the outside ectoderm (the lappets) and a great part of the ciliated band. veg_1 also contributes to the ciliated band and forms, moreover, the insides of the lappets, and the œsophagus. veg_2 constitutes the material for the stomach and perhaps a small part of the œsophagus as well.

V. The Position of the First Furrow

In order to interpret the results of isolating the first two blastomeres, it is necessary to know the position of the first furrow, whether it has a fixed position in relation to the median plane of the larva or may form any angle to this plane. In 34 2-cell stages one blastomere was stained by leaning it against a piece of agar. Owing to the large size of the blastomeres, the stain seldom penetrated the whole blastomere. Thus we did not get a very exact staining, with a sharp border line along a meridian of the egg. The stain rather indicated that side of the blastomere which was opposite the furrow. In 9 of the larvæ the color was too weak or too diffuse. In the other 25 the following parts of the larvæ were found to be blue: dorsal 5, ventral 3, left 6, right 3, dorsal-left 3, dorsal-right 4, ventral-right 1.

The number of cases is small and the method not very exact. Nevertheless, I think these results show that the first furrow is not confined to a certain plane, but probably may have any position in relation to the median plane. Isolating half-blastomeres, we shall thus obtain dorsal and ventral or right and left or oblique meridional halves.

VI. Isolation Experiments

A. Isolation of Animal and Vegetative Halves of Unfertilized Eggs

Wilson (1903), Zeleny (1904), and Yatsu (1904, 1910*a*) found that fragments from any part of the unfertilized *Cerebratulus* egg may be fertilized and may segment as whole eggs. Zeleny and Yatsu found that fragments obtained during the stages between the fertilization and the completion of the first cleavage show a progressive specification

of cleavage factors: just before initiation of the first cleavage the fragments segment as halves. Wilson followed the further development of fragments from unfertilized eggs and found that, if of sufficient size (not smaller than one-quarter), they may produce normally formed dwarf pilidia. According to Yatsu (1910*b*) even an animal half of an egg that has already given off the polar bodies is able to gastrulate and develop into a pilidium.

As the cleavage of fragments has been studied in such detail, I only wanted to repeat the experiments on the localization of gastrulation potencies in fragments of the unfertilized egg. Animal and vegetative fragments were isolated from the beginning of the fading of the germinal vesicle up to the formation of the first polar spindle (as far as the maturation proceeds before fertilization). The eggs used were not very satisfactory for they were the last of the season and the worms had lived for a long time in the ice-box. Eighteen animal and 20 vegetative fragments were fertilized. Five of the animal and 10 of the vegetative fragments died. The remaining 13 animal and 10 vegetative fragments all gastrulated. The best of the vegetative fragments developed into pilidia, whereas the animal ones died before that stage.

Our finding that not only the vegetative, but also all animal fragments gastrulate and that vegetative fragments may form apical organs supports Wilson's results (1903) that fragments from any part of the unfertilized egg are able to produce dwarf pilidia.

B. Isolation of Half-Blastomeres

Charles B. Wilson (1900) was the first to obtain two pilidia from one egg by isolating the half-blastomeres. E. B. Wilson (1903), Zeleny (1904) and Yatsu (1910*a*) found that the isolated half-blastomeres segment, not as wholes, but typically as if the missing blastomere were present, thus a strictly partial cleavage. In the following development, sometimes closed and spherical, sometimes cup-shaped or flat, plate-like forms were observed. All these types of young blastulæ could produce pilidia, but those from the plate-like forms were usually asymmetrical. The best-developed dwarf larvæ seemed to be typical in every respect. But some abnormalities were frequent among the other pilidia. The apical organ was sometimes displaced towards the anterior end (Wilson). However, Wilson never found this organ duplicated in half-larvæ. Zeleny observed no constant defect except possibly in the case of the lappets. But he generally killed his larvæ before the lappets could have developed fully. Yatsu (1910*b*) obtained some perfect pilidia, some with slight abnormalities, among them not infrequently double apical organs. He concludes

that bilaterality of egg substances cannot be detected at the 2- or 4-cell stage.

As the segmentation of half-blastomeres has been studied in detail by E. B. Wilson, Zeleny, and Yatsu, I restricted my observations to the differentiated larvæ. The two half-larvæ derived from one egg were reared together in order that the development of the bilateral symmetry in the pairs might be studied. The lappets were formed in four days.

Some larvæ developed into perfect dwarf pilidia. Others were more cylindrical in shape, with a short ciliated band, not forming any lappets. Some looked spherical from the side, namely when the main part of their bodies had the typical form, but the ciliated band was very short, forming only a narrow ring round the mouth. In some cases a lappet was formed on one side only. In many larvæ the apical organ was displaced towards the anterior end, as Wilson (1903) found, but a displacement to the posterior side was also observed. Contrary to Wilson's statement, in several cases I saw the apical organ doubled in the half-larvæ. Not only was the flagellum split up in two, but larvæ with two separated ectodermal pits were found.

If we now turn to the pairs, there were 6 pairs with both larvæ typical, symmetrical, thus each larva with two lappets (Fig. 2, *B*, *C*). The largest group, 13 pairs, is characterized by one typical and one cylindrical larva. In the latter the shape of the œsophagus-stomach generally indicates a bilateral symmetry, which is, however, hardly expressed in the ectoderm. In 6 cases both partners were cylindrical. In 3 pairs one larva was typical, the other irregular and difficult to interpret. One larva had a lappet only on the right side, the partner being cylindrical. In one pair the two pilidia showed supplementary deficiencies: one had a left, the other a right lappet. This is what would be expected if we had a rather definite bilateral organization of the egg in the 2-cell stage. But against this one case were two where both partners had the lappets better developed on the same side, the right.

In the sea urchin, Hörstadius and Wolsky (1936) have demonstrated that the larvæ derived from isolated half-blastomeres, from halves of the 16-cell stage, and even halves of the unfertilized egg, show characteristic differences, according to their origin from the left and right, or dorsal and ventral parts of the egg. In *Cerebratulus* I was not able to refer the half-larvæ to certain parts of the egg. We know that the larvæ obtained may correspond to right and left, or dorsal and ventral, or oblique meridional halves of the egg (*cf.* above). But only in one case of 29 did we find a bilateral asymmetry that would indicate right and left halves. At the same time there were two pairs

with the right side better developed in both partners, thus detracting from the value of the supplementary deficiencies in the first-mentioned case. Furthermore, I was not able to detect any features that would incline me to ascribe one partner to the dorsal, the other to the ventral part of the egg. There is no sign of the cylindrical larvæ arising from a dorsal, or ventral fragment. Thus the abnormalities in the cylindrical, rounded, or irregular larvæ do not, as far as I can judge, tell us anything about the origin of these larvæ. Our conclusion, then, would be like that of Yatsu, that the *Cerebratulus* egg in the 2-cell stage is not yet so markedly bilaterally organized that the half-larvæ show deficiencies on the side where material has been removed.

C. Isolation of Quarter-Blastomeres

The quarter-blastomere also undergoes a fractional cleavage analogous to that characteristic of the half-blastomere (Wilson, 1903;

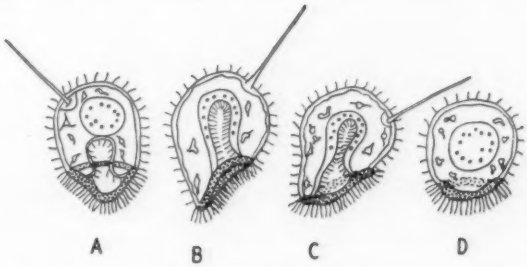


FIG. 4. Four larvæ from the isolated quarter-blastomeres of one egg.

Zeleny, 1904). It gives rise to a much larger proportion of abnormal forms, and even at best the larvæ were never normal in every respect (Wilson, 1903; Yatsu, 1910*b*). Zeleny (1904) killed his quarter-larvæ before they were fully differentiated. The apical organ was generally markedly displaced towards the anterior end (Wilson, Yatsu), or it might be lacking (Yatsu). The archenteron, also, could be abnormally formed, displaced towards the posterior end (Wilson).

Seven quartets of quarter-blastomeres were isolated, the blastomeres being reared in separate dishes and kept in such order of sequence as in the 4-cell stage, so that we know, for instance, that No. 4 had had Nos. 3 and 1 as neighbors. The larvæ were all more or less irregular (Fig. 4). The apical organ was generally displaced, or might be absent; the archenteron was not always well differentiated. The position of the ciliated band was in most cases more or less oblique. But, as in the half-blastomeres, it was not possible to find any definite

relation between these asymmetries and a supposed bilateral organization of the egg.

D. Isolation of the Four Animal and the Four Vegetative Cells of the Eight-Cell Stage

Zeleny (1904) found that larvæ developing from the upper quartet have an apical organ, but no archenteron, those from the lower quartet have an archenteron, but no apical organ, while those from lateral four-cell groups have both apical organ and archenteron. Zeleny drew the conclusion that certain organ-forming materials are definitely separated by the third furrow and that the larvæ from the upper and the lower quartet have not the power of replacing the material lacking. The meridional halves, however, possess both kinds

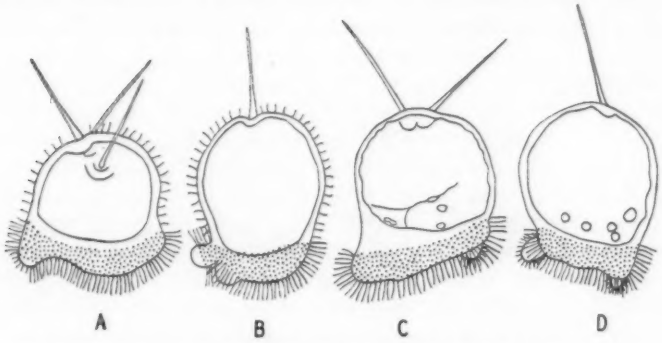


FIG. 5. Larvæ from the isolated four animal cells of the 8-cell stage.

of material. Zeleny preserved his larvæ before they were fully differentiated. Yatsu (1910*b*) isolated 7 animal quartets, six of which gave a blastula with apical organ, with 1 to 3 flagella, and, to judge from the drawings, a ciliated field or band at the opposite pole. Thus no gastrulation occurred. Strange to say, the seventh larva developed into an almost perfect pilidium (Yatsu, 1910*b*, Fig. 18, *D*). Of the 3 vegetative quartets Yatsu mentions two as "defective." The third is an almost normal pilidium (*loc. cit.*, Fig. 18, *I*).

My material numbers 10 pairs of animal and vegetative quartets, and moreover 9 animal and 4 vegetative fragments of the same kind, but of which the corresponding vegetative and animal quartet respectively are missing. None of the 19 animal fragments gastrulated. They gave a blastula with a pretrochal pavement epithelium, a thickened vegetative ciliated band or field, and one, or several, apical

organs (Fig. 5). Ten of these blastulæ had but one normal apical organ, 4 had two, and 3 had three flagella. In most cases, perhaps in all, every flagellum had its own ectodermal pit. In one case the apical organ was entirely lacking. One blastula was very peculiar, with seven flagella, three of them on the pretrochal part, the other four growing out from the ciliated band. The shape of the animal larvæ is shown in Fig. 5. It is rather rounded or ovoid, the ciliated band occupying the broader or the narrower end. The "ciliated band" has more the character of a ciliated field of high cells, with here and there some irregular protrusions. This ciliated area has the yellowish color of the ciliated band, and the cilia wave in the same typical way. Thus, on the whole, the animal quartet develops just as it would have done in the normal larva, giving rise to the same kind of tissues: pretrochal ectoderm with apical organ, and ciliated band tissue. A difference lies in the frequent duplication of the apical organ.

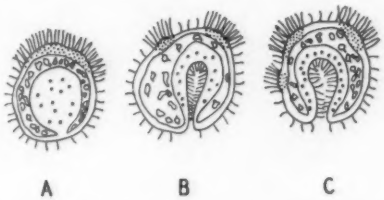


FIG. 6. Larvæ from the isolated four vegetative cells of the 8-cell stage.

The vegetative quartets also (Fig. 6) differentiate in accordance with their prospective significance. An archenteron invaginates, but it evidently corresponds only to the stomach of the pilidium, as it differentiates in the same way, containing the small crystals characteristic of the stomach. The "ectoderm" of the gastrula corresponds to the œsophagus and the insides of the lappets, and at the animal pole of this vegetative fragment we find a ciliated field, presumably derived from the material that normally contributes to the formation of the ciliated band. This field is, however, often divided into two or more ciliated patches of the same kind of tissue as the ciliated band.

All the animal and vegetative fragments thus developed in accordance with their prospective significance. When Yatsu (*cf.* above) found that one animal and one vegetative fragment developed into almost perfect pilidia, he must have isolated two meridional halves instead of one animal and one vegetative. The orientation of the 8-cell stage is not always easy, unless the polar bodies are clearly seen.

E. Isolation of an_1

In two cases Zeleny (1904) successfully separated the four animal cells of the 16-cell stage (an_1) from the twelve lower ($an_2 + veg_1 + veg_2$). The two an_1-larvæ possessed an apical plate but were not reared long enough to show the differentiation in detail.

Twenty-five an_1 were isolated. One did not develop very well. Fourteen disappeared altogether (*cf.* p. 319), and the remaining 10 developed into blastulæ with apical organs (Fig. 7). Five of them had one flagellum, 3 had two flagella, and 2 none. I had the impression that the pit in some cases was missing or not so pronounced as in normal pilidia, but this observation may be erroneous. It may be mentioned that Yatsu (1904), in the uncleaved egg, localized the basis

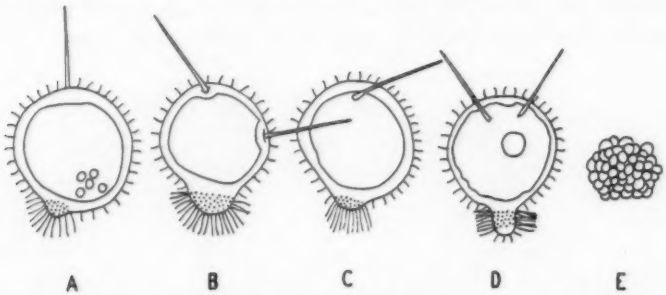


FIG. 7. *A–D.* Larvæ from the isolated four most animal cells of the 16-cell stage: an_1. *E.* Isolated veg_2 (the four most vegetative cells of the 16-cell stage).

of the apical organ, not in the animal pole, but in a broad zone a little above the equator. Wilson (1903) also observed flagella without ectodermal pit, namely in larvæ from vegetative egg fragments. There is need of further elucidation of the question whether the absence of the ectodermal pit in an_1-larvæ, if this takes place, has any relation to the localization suggested by Yatsu. If so, it would indicate a trace of dependent differentiation.

The ectoderm of these an_1-blastulæ was ciliated in the usual way. At the pole opposite the apical organ they had small fields of ciliated band tissue (high, yellowish cells). In some cases this field had the form of a protrusion, with the ciliated band like a girdle round it (Fig. 7, *D*). I interpret this to mean that the patch of ciliated band tissue in an_1 corresponds to that part of the normal band which, on the basis of our staining and transplantation experiments, we ascribed to the an_1-cells (See Fig. 3).

F. Isolation of $an_2 + veg_1 + veg_2$

The best one of two fragments of this kind that Zeleny obtained (*cf.* above) formed a ciliated rotating embryo, with a large solid archenteron filling up the cavity of the blastocœl. Neither apical organ nor lappets were present.

Of the five larvæ only one developed really well. In the early gastrula stage the archenteron seemed to fill up the entire blastocœl, as Zeleny found. Also in the early pilidium the archenteron looked very large in comparison to the ectoderm. A day later this was no longer the case to such an extent as one would have expected after removal of such a large part of the presumptive ectoderm (Fig. 8, *A*).

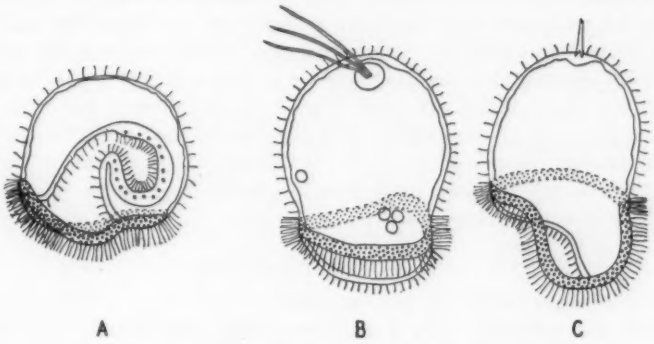


FIG. 8. *A*. $an_2 + veg_1 + veg_2$. *B*, *C*. $\bar{a}n_1 + an_2 + \bar{v}eg_1$.

The larva now appears as a small pilidium, save that the apical organ is missing. The stomach is of normal size. The œsophagus is smaller than normal but probably contains as much material as in the normal pilidium, as its wall is thicker. The ciliated band and the pretrochal ectoderm are too small, which is easily understood in that the larger part of the ectoderm has been removed. That, in spite of this, the ectoderm does not look still narrower is probably due to an extreme stretching and thinning of this material.

G. Isolation of veg_2

This region constitutes the material for the stomach (perhaps also a small part of the œsophagus). When isolated it does not live long. On the first day after fertilization these fragments were generally all dead. Those which lived the longest formed a mass of cells, as seen in Fig. 7, *E*. Only in one case were some cilia developed, indicating an initial differentiation.

H. Isolation of $an_1 + an_2 + veg_1$

Only three fragments of this type were studied, but they all developed in a similar way. They formed, much as the isolated four animal cells of the 8-cell stage, a blastula with an apical organ and a ciliated band (Fig. 8, *B*, *C*). Thus no real deep gastrulation took place, although the vegetative end of the larva at one stage may be a little curved inwards, as in a normal larva just at the beginning of the gastrulation. This part later differentiated into an epithelium which mostly looked like ectoderm, but perhaps was a little more densely ciliated. In the larva from the upper quartet of the 8-cell stage the "ciliated band" rather was a ciliated field of high cells, occupying the lower part of the larva (Fig. 5). Our present larvæ differ in the respect that we here have a real band, surrounding the ectoderm-like area just mentioned, which in turn must correspond to the inside of the lappets and the œsophagus. In one of the larvæ this area first curved inwards and was later turned out again (Fig. 8, *B*), whereas in the other two it remained as a very shallow invagination; the ectoderm formed like a lappet on one side (Fig. 8, *C*). Thus also a larva from which veg_2 has been removed seems to develop in accordance with the prospective significance of the material.

I. Isolation of $an_2 + veg_1$

The same holds for the fragments $an_2 + veg_1$. A great many larvæ disappeared. The 9 surviving ones differentiated, although varying in shape, into blastulæ with a broad, ciliated band around the equator (Fig. 9). As the ectoderm does not expand, the ciliated band

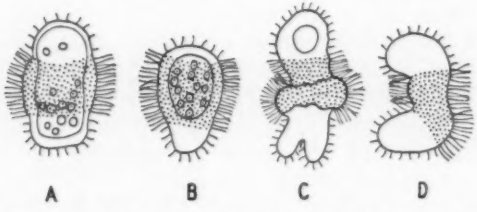


FIG. 9. Larvæ from the two middle layers of the 16-cell stage: $an_2 + veg_1$.

is very thick and broad. The pretrochal, animal epithelium which corresponds to the thin ectoderm formed by an_2 is generally thinner than the vegetative epithelium of the band (corresponding to the inside of the lappets and the œsophagus). Some larvæ are more or less massive, and irregular in shape (Fig. 9, *C*, *D*).

VII. Transplantation Experiments

A. $an_1 + veg_2$

The most animal and the most vegetative of the cell layers of the 16-cell stage were fused for the purpose of studying whether organs that normally arise from the middle part of the egg could be formed by regulation. The larvæ gastrulated, the veg_2-material being invaginated. The ectoderm appears identical with that of the isolated an_1, having an apical organ and a small patch of ciliated band tissue (*cf.* Figs. 10 and 7). In some cases the archenteron differentiated into just a stomach, very characteristic with its crystals in the wall. But in other cases I also obtained what looked like a small part of an œsophagus (Fig. 10, *C*). This might be a structure formed by regulation,

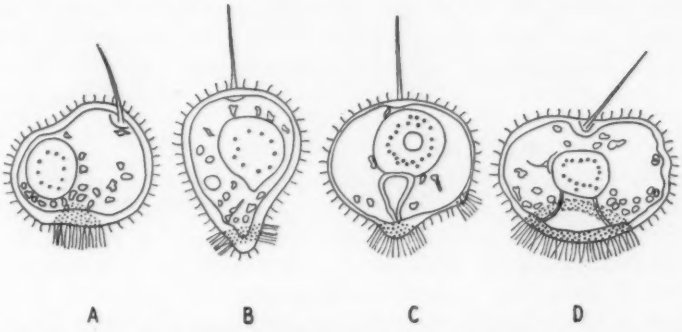


Fig. 10. *A–C.* Larvæ composed of the most animal and the most vegetative layers of the 16-cell stage: $an_1 + veg_2$. *D.* $an_1 + \frac{an_2}{2} + veg_2$.

but, as pointed out on p. 321, it is possible that veg_2 normally also forms a part of the œsophagus. I am not sure that the third furrow always lies at the same level. I have the impression that the differences in size between the animal and the vegetative cells of the 8-cell stage may vary. As a consequence probably the cleavage plane veg_1–veg_2 also may vary in relation to the limit between the presumptive œsophagus and presumptive stomach. This would explain the slightly different results (Fig. 10, *A–C*).

B. $an_1 + \frac{an_2}{2} + veg_2$

In three cases veg_2 was added to $an_1 + 2\ an_2$-cells. This increase in ectoderm and ciliated band material gave a larva of essentially the same type as $an_1 + veg_2$, but with a larger ectoderm and a larger amount of ciliated band tissue, which now forms a complete ring (Fig. 10, *D*).

C. Fusion of an Animal and a Meridional Fragment of the Eight-Cell Stage

The problem is whether the entoderm material of a meridional fragment of the 8-cell stage can bring about any entodermization of adjacent presumptive ectoderm from an animal fragment, and whether a regulation will take place also regarding other organs (apical organ, ciliated band), so that a harmonic individual arises. The animal fragment of the 8-cell stage was vitally stained before transplantation. At the fusion the polarity of this fragment stands at right angles to that of the meridional one (Fig. 11, *A*).

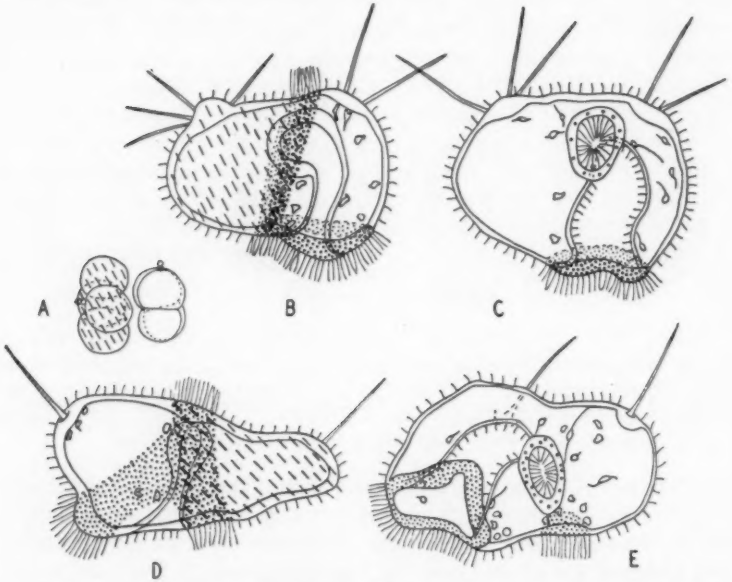


FIG. 11. *A*. Fusion of a meridional half and a vitally stained (stippled) animal half of the 8-cell stage. *B, D*. The larvæ have differentiated conformably to their prospective significance. *C, E*. Commencing regulation by redifferentiation.

The 5 larvæ all gave the same result (Fig. 11, *B, D*). Each component differentiated in the same way as it would have done normally; there seemed to be no interaction between them whatsoever. At gastrulation none of the adjacent blue animal material was invaginated. The meridional partner developed an apical organ at its animal pole and half of a ciliated band, embracing the mouth like a horseshoe. The animal component clearly has a polarity at right angles to that of the meridional one. The apical organ of the animal fragment is situated

at its animal pole; its ciliated band forms a girdle round its base, at right angles to the horseshoe of the meridional component. In the larva, Fig. 11, *D*, the apical organ of the meridional fragment is not situated close to the stained ciliated band. The position of the two ciliated bands gives the explanation. They are not at right angles to each other. The fusion of the two components has occurred obliquely.

Thus both halves differentiated according to their prospective significance. It is now highly interesting to note that, in spite of the lack of interaction in the period of embryological differentiation, we find a regulative interaction in the following development. Several days after the differentiation of the organs of the pilidium, the ciliated band of the animal component gradually disappeared. Only a short part of it remained to form, together with the horseshoe of the meridional part, a complete ciliated ring (Fig. 11, *C*; *cf.* also *E*). Thus we here witness a step towards the individualization of this heterogeneous larva by means of redifferentiation. The larvæ did not live long enough to show any further changes.

VIII. Conclusions and Discussion

By means of vital staining the prospective significance of the animal and the vegetative cells of the 8-cell stage, and of the four cell layers an_1, an_2, veg_1, and veg_2 of the 16-cell stage, was studied. The isolation experiments showed that, on the whole, the fragments isolated by cuts vertical to the egg-axis differentiated in accordance with the prospective significance of such parts interpreted through the presence of different organs, e.g., apical organ, ectoderm pavement epithelium, ciliated band, epithelium of the inside of the lappets, œsophagus, and stomach. There is some doubt, however, as to whether all fragments differentiate in every detail according to their prospective significance, as the latter could not be determined with accuracy in all cases. The staining experiment was not quite conclusive as to the fate of an_1, but the transplantation experiment (Fig. 3), too, indicates that an_1 normally forms a piece of the ciliated band, as the isolated fragment always does (Fig. 7). It is more uncertain whether veg_2 normally contributes to the œsophagus. We must thus leave open the question whether the small œsophagus in some of the larvæ $an_1 + veg_2$ (Fig. 10) was formed from presumptive œsophagus or by regulation. The rather extensive ectoderm in the larva $an_2 + veg_1 + veg_2$ may result from an unusual stretching of the material. The mosaic development of the larvæ in Fig. 11 supports the view that the apparent deviations from the prospective significance in the differentiation of the cases just mentioned are more apparent than real.

It was suggested as possible that the apical organ is not definitely determined in an_1. An interesting feature is the appearance of several apical organs in fragments (not only is the flagellum subdivided but there may also be several ectodermal pits). Is that a sign that the organ was not determined at the time of operation? Not necessarily, as it may, on the contrary, indicate a mosaic differentiation. If the apical organ is normally formed from all four quadrants, a slight relative change of position of the animal parts of the blastomeres at or after the operation may cause the parts of the presumptive apical organ to become separated by other ectoderm. In my experiments I never got more than two apical organs in half-larvæ, only one in the quarter-larvæ, and, with one exception, not more than two or three in animal fragments, thus never more than the number of quadrants present. On the other hand, the exception, an animal fragment with seven separate flagella (p. 327) seems to show, as well as the possible lack of ectodermal pit in an_1-larvæ (see p. 328), that the determination of the apical organs in fragments is more complicated. The problem must be left unsolved.

The scope of this investigation was to compare an egg with mosaic development with that of the sea urchin. An animal fragment of the sea urchin egg (an_1, or $an_1 + an_2$ of so-called equatorial eggs (Hörstadius, 1935, p. 309)) forms less organs (no ciliated band, no stomodæum) than it would have done in normal development, whereas a vegetative half, by regulation, often forms more organs than the same material would normally have yielded (the vegetative halves may have apical tuft, mouth, oral arms). An isolated veg_2-layer develops into a larva with both more animal (ectoderm) and more vegetative differentiations (skeleton) (*loc. cit.*, p. 423) than it would have given rise to in normal development (archenteron). Another way of demonstrating the changes of differentiation that may occur in the sea urchin is to add a vegetative fragment to an animal fragment, e.g., to add the four micromeres to an_1 or to an animal half ($an_1 + an_2$). As a result of an interaction between the animal and the vegetative qualities a complete dwarf pluteus develops (*loc. cit.*, p. 330).

Our experiments with *Cerebratulus* have shown that there is probably no change in differentiation in animal and vegetative fragments of cleavage stages. If any changes take place they are so slight that we have not been able to detect them with certainty. At all events, there is no regulation to compare with that of a vegetative half, or a veg_2, in the sea urchin, and, in the *Cerebratulus* fragments, there is no failure of some organs to appear (in comparison to the prospective significance) as in the isolated an_1 or $an_1 + an_2$ of the sea urchin. We have several

directly comparable experiments which show this very clearly. In the sea urchin it is possible to obtain two plutei from one egg, after cutting at right angles to the egg-axis, i.e., if we cut twice and put the two polar parts together (Hörstadius, 1936*a*). The middle part will thus give more animal and more vegetative differentiations than it would have done normally, the animal and vegetative fragments (an_1 + the micromeres, or $an_1 + an_2$ + the micromeres) will, by regulation, form the organs characteristic of the excised middle part of the egg (archenteron, etc.). The equivalents to these experiments are our isolations of $an_2 + veg_1$ (Fig. 9) and fusion of $an_1 + veg_2$ (Fig. 10) in *Cerebratulus*. As far as we can judge, these larvæ show mosaic development. The second experiment implies the adding of an animal half to a meridional half, at right angles to each other as regards polarity. This larva differentiates in the sea urchin into a perfect pluteus. The animal half becomes completely incorporated: a part of it is entodermized, now taking part in the formation of the archenteron, and the ectoderm, too, acts in all details (ciliated band, stomodæum) as a part of the new individual (Hörstadius, 1928, 1935). In *Cerebratulus* this is not true (Fig. 11). We find a strict mosaic development, without any trace of entodermization or accommodation of the ectoderm of the animal fragment to that of the meridional half.

I recall the fact that the ciliated band of the animal component of these larvæ may disappear. We witness an initial change of the heterogeneous larva into a more harmonic individual. It has already been pointed out (p. 333) that this change is brought about by a redifferentiation after the embryological differentiation is already complete. In this connection we must mention a similar observation by Yatsu (1910*b*). Whereas an apical organ is not formed in larvæ which in the cleavage stages were deprived of their most animal part, Yatsu found that the apical organ regenerated, when it was removed from late gastrulæ or young pilidia.

We have to remember that the mosaic development of animal and vegetative parts mentioned above concerns the layers of the 8- and 16-cell stages. Our isolation of animal and vegetative halves prior to fertilization confirms the results of Wilson (1903) and Yatsu (1910*b*), —that any fragment of the unfertilized egg may develop into a pilidium. Thus the transition from a regulation to a mosaic type takes place between the beginning of maturation (before fertilization, *cf.* p. 322) and the 8-cell stage. Yatsu (1910*b*) observed that an animal half of even a mature egg could form a pilidium. Removal of the most animal part of the egg in the first cleavage of the two blastomeres of the 2-cell stage had no effect, but when the most vegetative material

was cut off, the archenteron was missing or too small (Yatsu). Thus at that time the localization of the presumptive entoderm seems to have advanced a great deal. When 2-cell stages were compressed so that the second furrow came to lie equatorially, instead of meridionally, and the two animal cells were separated from the vegetative, the former did not gastrulate, whereas the vegetative gastrulated but formed no apical organ. Thus already at the 4-cell stage we seem to have exactly the same animal-vegetative localization as in the 8-cell stage.

Wilson (1903) and Zeleny (1904) have assigned the location of the entoderm material to the vegetative part of the egg and the determination of the apical organ to the rearrangement of material at the breaking down of the germinal vesicle. Yatsu (1904) observed, in sections, that a segregation of egg material does actually take place at that period, the yolk accumulating in the lower hemisphere while the clear and more finely granulated protoplasm collects at the animal pole of the egg. Some of the results presented by Yatsu as to the time at which determination has taken place are contradictory. Further investigations are desirable as to how much the determination becomes fixed along with the visible rearrangement of materials, and how much is a progressive process going on after the completion of this rearrangement, and hence a process of a different character.

The rearing of fragments of blastulæ has given startling results. It would seem as if these fragments were richer in potencies than fragments of cleavage stages. Wilson (1903), Zeleny (1904) and Yatsu (1910*b*) found that animal halves of blastulae could gastrulate, although the archenteron was generally smaller than that of the corresponding vegetative fragment. This result is in conflict with everything we know of the progressive embryological determination which leads to gradual restriction of potencies in every zone. (We recall the fact that the regeneration of an apical organ in the young pilidium and the redifferentiation in our larvæ meridional half + animal half took place only after the completion of the embryological differentiation—*cf.* p. 335.) Wilson is inclined to explain at least some of his results on the grounds of oblique sections. In view of this, Zeleny (1904) took special care in determining the orientation, but nevertheless he found in his two cases that the blastula fragments had greater regulative power than those of the 8- and 16-cell stages. One of Yatsu's animal fragments had a small gut, and the vegetative was provided with an apical organ. Until these results have been confirmed by means of very careful experiments, I am, like Wilson, inclined to explain them on the grounds of oblique sections. It is much more difficult to cut a blastula in a desired plane, than a cleavage

stage. In the latter you follow a furrow all through the egg. Moreover, if you start cutting a blastula equatorially with the correct orientation, the result may be oblique halves, for the knife may pass obliquely in animal or vegetative direction through the egg. This can only be detected by a careful examination of the fragments from all sides. The variability of the results on blastulæ, and the uniformity of those on 8- and 16-cell stages speak in favor of this assumption. As the fragments of the uncleaved egg are more or less equipotent, oblique cutting is most readily detected in the blastula stage.

If we turn to the fragments in which all the layers are present in the same proportion as in the egg (isolated half and quarter-blastomeres), we find that they are able to develop into more or less typical pilidia. All the organs are present, but the larvæ may be more or less asymmetrical, especially the quarter-larvæ. On the other hand, several pairs of half-larvæ showed a perfect bilateral symmetry (Fig. 2, *B*, *C*). These pairs probably correspond to both right-left, dorsal-ventral, and obliquely separated meridional halves (*cf.* p. 322). Furthermore, the pairs with asymmetrical larvæ did not show from which parts of the egg the halves originated. This seems to indicate that the bilateral symmetry is not fixed at an early cleavage stage when the animal-vegetative layers are already determined. In this respect a comparison with the sea urchin is of great interest. It has not been possible to study experimentally the unripe egg of the sea urchin. In the mature egg, before fertilization, we find not only an animal-vegetative segregation of presumptive ecto- and entoderm, but also slight traces of a bilateral organization. These traces are more marked in the cleavage stages. Right and left halves show complementary deficiencies of the skeleton on the cut side. Ventral halves develop their ventral side faster and better than dorsal halves. It is very interesting that the dorso-ventral axis in the dorsal halves is inverted (Hörstadius and Wolsky, 1936). I regret that time did not allow me to study in a similar way (by staining the cut side of the two isolated half-blastomeres) the relation of the median planes of the half-larvæ to that of the egg of *Cerebratulus*.

Thus the animal-vegetative determination in *Cerebratulus* seems to take place between fertilization,—at the beginning of maturation,—and an early cleavage stage. In the sea urchin we find a localization of presumptive ecto- and entoderm already in the mature, unfertilized egg but the same degree of determination is not reached as in the 8-cell stage of *Cerebratulus* until much later; in the case of some organs not until the beginning of gastrulation (Hörstadius, 1936*b*). On the other hand, the unfertilized, but mature sea urchin egg already seems to have a bilateral organization, which can be traced in meridional frag-

ments, whereas a bilateral organization of the *Cerebratulus* egg is not possible to detect, even in the early cleavage stage. It has many times been pointed out in the literature that there is no fundamental difference between the mosaic and the regulation eggs. In some eggs the determination sets in at an earlier stage than in others. This relative displacement in time is strikingly illustrated in these cases. In the *Cerebratulus* egg the animal-vegetative determination is accomplished much earlier; the determination of the bilateral symmetry later than in the sea urchin egg.

The formation of mesenchyme has not yet been mentioned. It would have been of great interest to determine whether, in fragments, mesenchyme can be budded off from other parts of the egg than those under normal conditions. In the figures, mesenchyme cells have been drawn in those larvæ in which they were clearly seen. In many isolated fragments we find cells in the blastocœl which are not real mesenchyme cells, but pathological. Those are more rounded, and often larger than the true mesenchyme cells. We find typical mesenchyme cells in the half- and quarter-larvæ (Figs. 2 and 4), in the isolated vegetative cells of the 8-cell stage (Fig. 6), in $an_1 + veg_2$ (Fig. 10), and in the meridional half + the animal fragment (Fig. 11). Many of the an_1 (Fig. 7, *B*, *C*) and the $an_1 + an_2$-larvæ (Fig. 5, *A*, *B*) were empty, others had some cells of the pathological type in the blastocœl (Fig. 7, *A*, *D*, 5, *C*, *D*). Only in one case was just one single cell observed which looked like a true mesenchyme cell (Fig. 5, *C*). The $an_2 + veg_1$-larvæ had some cells in their interior, but I could not determine their nature. To summarize, the larvæ with veg_2 present show typical mesenchyme cells (Figs. 2, 4, 6, 8*A* cells not drawn, 10, 11). The animal fragments (Figs. 5 and 7) have with one exception no typical mesenchyme cells. As regards the rôle of veg_1, we cannot say anything with certainty. The character of the cells in Fig. 9 ($an_2 + veg_1$) is unknown, and as to the larvæ $an_1 + an_2 + veg_1$ (Fig. 8, *B*, *C*), I have no records regarding the mesenchyme cells. Even with more detailed observations on the occurrence of the mesenchyme in fragments, it would be difficult to state whether the prospective potency as regards mesenchyme formation exceeds the prospective significance, as observations on normal mesenchyme and mesoderm formation are varied and contradictory. Coe (1899) traces the mesenchyme to the divisions of a large posterior pole cell, as in annelids, and to some of the entoderm cells. Charles B. Wilson (1900) speaks of micro- and macromesencytes, both derived from large entoderm cells close to the ectoderm. E. B. Wilson (1903) describes two symmetrically placed mesoblast cells which, just before invagination, pass into the cleavage cavity near one end of the embryo, and from them smaller mesenchyme

cells are budded forth, without, however, giving rise to definite mesoblast bands, as in the annelid embryo. E. B. Wilson finds it probable that the first two mesoblast cells do not arise from a single cell. All these statements refer to the genus *Cerebratulus*. Nusbaum and Oxner (1913) compare the observations of previous investigators with their own and come to the conclusion that the mesenchyme in the genus *Lineus* has a double origin: "(1) Aus Mesoblastzellen, die sich sehr früh aus den Mikromeren des vierten Quartetts differentieren und (2) aus nachträglich schon im Gastrulastadium aus dem Entoderm sich abtrennenden Zellen." According to Nusbaum and Oxner (1913) the micromere 4d in *Lineus* divides into two teloblasts (Urmesodermzellen), whereas Lebedinsky (1897), in *Tetrastemma* and *Drepanophorus*, speaks of four mother cells for four mesoblast bands. In *Malacobdella* Hammarsten (1918) also traces the mesoderm to four mother cells, 2a–2d. Thus the observations diverge widely, even within one genus, and even within one species. A renewed study of the mesenchyme formation in *Cerebratulus*, combined with isolation experiments, would be of great value. One problem is whether veg_1 normally and in fragments buds forth any mesenchyme. As half-larvæ (which may be right-left as well as dorsal-ventral halves) and quarter-larvæ always have mesenchyme cells, a second problem is whether the mesenchyme normally arises in all four quadrants of the egg, or the mesenchyme in some of the meridional fragments is formed by regulation. It may be added that the mesenchyme of the pilidium corresponds to both the larval mesenchyme (ectomesoderm) and the entomesoderm of the trochophore, as a part of the mesenchyme cells in the pilidium preserve an indifferent character until the formation of the worm.

It would lead us too far to consider here all experiments on other eggs with spiral cleavage. A full review of the literature on annelids and mollusks has been given by Schleip (1929); see also Huxley and de Beer (1934, Chapter V). The main difference between the nemertean egg and those of the other groups is the equality of the four quadrants of the egg in the former. In the uncleaved eggs of annelids and mollusks we are dealing with specific substances, more or less visible, which are of an "organ-forming" nature, the pole-plasms. They are precociously chemo-differentiated (Huxley and de Beer) to varying degrees in different species, and, moreover, the time at which they become specifically localized varies. Very often a polar lobe is formed temporarily during the first cleavage divisions. Removal of this lobe leads to an absence of the apical organ and to a defective post-trochal region; no somatoblasts will be formed. Because of this the isolated quarter-blastomeres develop in a very different way. Only the D-quadrant—the one that has the polar lobe—can produce somatoblasts

(which give rise to the ectodermal and mesodermal (entomesoderm) germ-bands. The A-, B-, and C-blastomeres cannot develop into complete larvæ. They may have larval mesenchyme (ectomesoderm), but are devoid of entomesoderm. As regards the apical organ, the conditions are, however, not so uniform. In *Patella*, for example, (Wilson, 1904*b*) all four quadrants acquire an apical organ, in *Dentalium* (Wilson, 1904*a*) only the D-quadrant, whereas in *Sabellaria* (Hatt, 1932) the factor determining the apical organ goes to the C-quadrant.

In mosaic development we thus find (at varying times) precociously differentiated substances distributed to particular regions of the egg, and in varying degrees by different forms, and subsequently isolated by the cell divisions. If the vegetative pole-plasm be equally distributed to the first two cells (*Tubifex*, by heat or deprivation of oxygen, Penners, 1924; *Chætopterus*, *Nereis*, and *Cumingia* by compression, high or low temperature, centrifuging, or anaerobiosis, Titlebaum, 1928, Tyler, 1930) double monsters are formed, or the blastomeres may, if isolated, give rise to more or less complete larvæ. In isolated normal half-blastomeres we thus find a self-differentiation in AB, on the one hand, differing from that of CD on the other, but when the vegetative pole-plasm is evenly distributed, both are capable of producing all organs. An interesting problem still remains unsolved. The isolated normal blastomeres differentiate as fragments, but would the same blastomeres behave in the same way if placed in contact with each other atypically? Although the "organ-forming" substances are now separated by cell-walls, could any kind of interaction be detected, any kind of change of the prospective significance of the material be brought about? Penners (1926, 1934) obtained, after killing teloblasts of *Tubifex* at different stages, a development in many respects of mosaic character, but also detected a certain dependence of the ectodermal and mesodermal components of the germ-bands on each other, and on the entoderm of the germ-bands, etc.

In *Cerebratulus* we also have an early rearrangement of substances, namely at the time of the breakdown of the germinal vesicle. But these substances are not, as far as we know, unevenly distributed to the quadrants. As has been pointed out above, we do not yet know with certainty or in detail at what time the progressive animal-vegetative determination is accomplished. The potencies of the blastula are still particularly obscure. Nor do we know how much of that determination is due to a rearrangement of substances, or to another metabolic process. A close study of mesenchyme formation in *Cerebratulus*, both in normal development and in animal-vegetative and meridional fragments, would be of special interest in rendering possible a comparison with the conditions in annelids and mollusks.

IX. Summary

1. The prospective significance of the animal and the vegetative cells of the 8-cell stage, and of the layers an_1, an_2, veg_1, and veg_2 of the 16-cell stage (Fig. 1) was studied. an_1 forms the greater part of the pretrochal ectoderm, including (probably) the most anterior part of the ciliated band (Fig. 3). an_2 gives rise to the rest of the pretrochal ectoderm, and a great part of the ciliated band. veg_1 also partakes in the formation of the ciliated band, and, moreover, differentiates into œsophagus and the insides of the lappets. veg_2 corresponds to the stomach. The boundary between the layers could not, however, always be determined with complete accuracy.

2. The first furrow may form any angle to the median plane of the larva.

3. The two isolated half-blastomeres from one egg may develop into pilidia with perfect bilateral symmetry (Fig. 2 *B*, *C*). When the isolated half- and quarter-blastomeres are less typical, their abnormalities do not show from which part of the egg the dwarf larvæ come. The bilateral symmetry is not determined in the early cleavage stage.

4. Animal and vegetative fragments differentiate, as far as we can judge from comparison with our results concerning the prospective significance, in the same way as they would have done in normal development: the animal (Fig. 5), and the vegetative (Fig. 6) cells of the 8-cell stage; an_1 (Fig. 7, *A–D*); veg_2 (Fig. 7, *E*); $an_2 + veg_1 + veg_2$ (Fig. 8, *A*); $an_1 + an_2 + veg_1$ (Fig. 8, *B*, *C*); $an_2 + veg_1$ (Fig. 9).

5. Larvæ composed of $an_1 + veg_2$ did not form any of the organs normally derived from the excised middle part of the egg (Fig. 10). Fusion of an animal with a meridional half of the 8-cell stage also showed a complete mosaic development (Fig. 11).

6. The results are discussed and compared with those with sea urchins, annelids and mollusks. The desirability of further investigations is emphasized.

LITERATURE CITED

Coe, W. R., 1899. The maturation and fertilization of the egg of Cerebratulus. *Zool. Jahrb., Anat.*, **12**: 425.

Driesch, Hans, 1891. Entwickelungsmechanische Studien. I. *Zeitschr. f. wiss. Zool.*, **53**: 160.

Hammarsten, Olof D., 1918. Beitrag zur Embryonalentwicklung der Malacobdella grossa (Müll.). Inaugural-Dissertation Stockholm. Uppsala 1918: 1. Also in *Arbeten fr. Zootomiska Institutet, Stockholm*, **1**.

Hatt, Pierre, 1932. Essais expérimentaux sur les localisations germinales dans l'oeuf d'un Annélide (Sabellaria alveolata L.). *Arch. d'Anat., Micr.*, **28**: 81.

Hörstadius, Sven, 1928. Über die Determination des Keimes bei Echinodermen. *Acta Zoologica, Stockholm*, **9**: 1.

Hörstadius, Sven, 1931. Über die Potenzverteilung im Verlaufe der Eiachse bei Paracentrotus lividus Lk. *Arkiv. Zool., Stockholm*, **23**: No. 1.

HÖRSTADIUS, SVEN, 1935. Über die Determination im Verlaufe der Eiachse bei Seeigeln. *Pubbl. Stazione Zoologica, Naples*, **14**: 251.

HÖRSTADIUS, SVEN, 1936*a*. Weitere Studien über die Determination im Verlaufe der Eiachse bei Seeigeln. *W. Roux' Arch. Entw.-mech.*, **135**: 1.

HÖRSTADIUS, SVEN, 1936*b*. Über die zeitliche Determination im Keim von Paracentrotus lividus Lk. *W. Roux' Arch. Entw.-mech.*, **135**: 1.

HÖRSTADIUS, SVEN, AND ALEXANDER WOLSKY, 1936. Studien über die Determination der Bilateralsymmetrie des jungen Seeigelkeimes. *W. Roux' Arch. Entw.-mech.*, **135**: 69.

HUXLEY, JULIAN S., AND G. R. DE BEER, 1934. The Elements of Experimental Embryology. Cambridge University Press.

LEBEDINSKY, J., 1897. Beobachtungen über die Entwicklungsgeschichte der Nemertinen. *Arch. mikr. Anat.*, **49**: 503.

NUSBAUM, J., AND M. OXNER, 1913. Die Embryonalentwicklung des Lineus ruber Müll. *Zeitschr. wiss. Zool.*, **107**: 78.

PENNERS, A., 1924. Experimentelle Untersuchungen zum Determinationsproblem am Keim von Tubifex rivulorum Lam. I. *Arch. mikr. Anat. u. Entw.-mech.*, **102**: 51.

PENNERS, A., 1926. Experimentelle Untersuchungen zum Determinationsproblem am Keim von Tubifex rivulorum Lam. II. *Zeitschr. f. wiss. Zool.*, **127**: 1.

PENNERS, A., 1934. Experimentelle Untersuchungen zum Determinationsproblem am Keim von Tubifex rivulorum Lam. III. *Zeitschr. f. wiss. Zool.*, **145**: 220.

SCHLEIP, WALDEMAR, 1929. Die Determination der Primitiventwicklung. Leipzig, Akad. Verlags.

TITLEBAUM, A., 1928. Artificial production of Janus embryos of Chaetopterus. *Proc. Nat. Acad. Sci.*, **14**: 245.

TYLER, A., 1930. Experimental production of double embryos in annelids and mollusks. *Jour. Exper. Zoöl.*, **57**: 347.

VON UBISCH, L., 1925. Entwicklungsphysiologische Studien an Seeigelkeimen, I. *Zeitschr. wiss. Zool.*, **124**: 361.

VON UBISCH, L., 1936. Über die Organisation des Seeigelkeims. *W. Roux' Arch. Entw.-mech.*, **134**: 599.

VOGT, WALTHER, 1923. Morphologische und physiologische Fragen der Primitiventwicklung, Versuche zu ihrer Lösung mittels vitaler Farbmarkierung. *Sitz.-ber. d. Ges. f. Morph. u. Physiol. München*, **35**: 22.

VOGT, WALTHER, 1925. Gestaltungsanalyse am Amphibienkeim mit örtlicher Vitalfärbung. I. *Arch. Entw.-mech.*, **106**: 542.

WESTBLAD, EINAR, 1923. Zur Physiologie der Turbellarien. *Kungl. Fysiograf. Sällsk. i. Lund Handlingar, N. F.*, **33**: No. 6.

WILSON, CHARLES B., 1900. The habits and early development of Cerebratulus lacteus (Verrill). *Quart. Jour. Micros. Sci.*, **43**: 97.

WILSON, EDMUND B., 1903. Experiments on cleavage and localization in the nemertine egg. *Arch. Entw.-mech.*, **16**: 411.

WILSON, EDMUND B., 1904*a*. Experimental studies on germinal localization. I. The germ-regions in the egg of Dentalium. *Jour. Exper. Zoöl.*, **1**: 1.

WILSON, EDMUND B., 1904*b*. Experimental studies on germinal localization. II. Experiments on the cleavage-mosaic in Patella and Dentalium. *Jour. Exper. Zoöl.*, **1**: 197.

YATSU, N., 1904. Experiments on the development of egg fragments in Cerebratulus. *Biol. Bull.*, **6**: 123.

YATSU, N., 1910*a*. Experiments on cleavage in the egg of Cerebratulus. *Jour. Coll. Sci. Tokyo*, **27**: No. 10.

YATSU, N., 1910*b*. Experiments on germinal localization in the egg of Cerebratulus. *Jour. Coll. Sci. Tokyo*, **27**: No. 17.

ZELENY, CHARLES, 1904. Experiments on the localization of developmental factors in the nemertine egg. *Jour. Exper. Zoöl.*, **1**: 293.

PROGRAM AND ABSTRACTS OF SCIENTIFIC PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY

JULY 6, 1937

Parthenogenetic merogony in the Naples sea-urchins. Ethel Browne Harvey.

Parthenogenetic merogony, or development of non-nucleate parts of eggs which have been artificially activated, has been studied in the four species of sea-urchin commonly occurring at Naples. The non-nucleate fractions were obtained, as previously, by breaking the eggs apart with centrifugal force.

In *Arbacia pustulosa*, which is similar to the *Arbacia* at Woods Hole, asters and cleavages occur in the non-nucleate half; when activated, similar to what has been described for *Arbacia punctulata*.

In *Parechinus* (*Echinus*) *microtuberculatus* and *Paracentrotus* (*Strongylocentrotus*) *lividus*, the fertilization membrane in activated non-nucleate halves is well separated from the cell surface, just as in normally fertilized whole eggs of these species. A large monaster is formed, an amphiaster and there are one or two cleavages. The cleavage planes frequently disappear and some time afterward the egg breaks up into a number of pieces. These become progressively smaller and more numerous until what resembles a blastula is formed. This breaks through the fertilization membrane, but no further development has been observed. The non-nucleate halves of *Sphaerechinus granularis* can also be activated and they break up into small pieces in a similar way.

It might appear that this breaking up of the egg is a form of degeneration or cytolysis. But the same spontaneous breaking up of the cell has been observed also in normal whole nucleate eggs artificially activated. These sometimes gave rise to typical blastulae which became free swimming and looked normal in every respect.

It has also been found that by treating the immature egg with parthenogenetic agents, there is formed a very definite layer on the periphery of the cell similar to the ectoplasmic layer in the fertilized or activated mature egg. This is formed both on the half containing the germinal vesicle and the heavier half separated from it by centrifugal force. Another evidence of activation is the failure to respond to sperm by the formation of blisters on the surface. The treated, immature egg, after some time, pinches off a small piece, always at the part most distant from the germinal vesicle in elongated eggs. Later there are two, three or more pieces and then a great number.

A recent critical examination of prepared slides of the parthenogenetic merogones of *Arbacia punctulata* shows the presence of asters, usually in pairs, but no chromosomes. The Feulgen reaction, in the procedure of which I was assisted by Dr. Jean Brachet, is entirely negative. The parthenogenetic merogones show no red-staining nuclear material, whereas the fertilized merogones which were used as control material, show it very clearly.

Some oxidative properties of isolated amphibian germinal vesicles. Jean Brachet.

Amphibian germinal vesicles isolated from full-grown oöcytes in a saline solution absorb oxygen and eliminate carbon dioxide during several hours; the

oxygen consumption was measured by means of a modified Garard-Hartline micro-respirometer using *Triturus pyrrhogaster* as material while the CO_2 excretion of *Rana fusca* nuclei was estimated by a titrimetric micro-method. The QO_2 $\frac{\text{(cu. mm. } O_2\text{)}}{\text{grams wet weight} \times \text{hour}}$ of the isolated nuclei (the approximate wet weight being calculated from the volume and the density) is about 13 while the full-grown oöcytes have a QO_2 of 37. The respiration of one single isolated nucleus is only 1–1.5 per cent of the amount of gas exchanged by one full-grown oöcyte deprived of its follicular epithelium. Removal of the germinal vesicle does not affect markedly the CO_2 elimination of full-grown oöcytes during several hours. Addition to the nuclei of glucose or of cytoplasm removed from the oöcytes with a micropipette does not increase their respiration. Presence or absence of Ca ions (which, according to W. R. Duryee, greatly affect the physical properties of the germinal vesicle) does not affect appreciably the metabolic rate of the isolated nuclei.

Influence of temperature and other agents on the respiration and development of marine eggs. Albert Tyler. (Most of the data given in this report appear in an article on p. 261 of this issue of the Biological Bulletin under the title, "On the energetics of differentiation, VI." The report has also been abstracted in the Collecting Net of July 10.)

JULY 13

Effects of fatigue due to muscular exercise on the Purkinje cells of the cerebellum of mice at various ages. Warren Andrew.

The problem of morphological changes in nerve cells as the result of functional activity has been studied by a number of investigators. Nevertheless, the question still remains open not only as to what changes occur due to activity but as to whether any such changes do occur.

Among the factors to be taken into account in such work is that of age. The present experimental work is based on earlier work in which the Purkinje cells of mice and rats were studied from the time of their differentiation up to and including extreme senility of the individual.

Sixteen black mice were used in the experimental work on fatigue. "Fatigue" means complete exhaustion brought about by running in a motor-driven rotary cage. For each fatigued animal, a control animal of the same brood was killed at the same time and the tissues from the two animals carried through the technical processes together.

The animals represent a range of ages including pairs of 23 days, 25 days, 43 days, 46 days, 98 days, 101 days, 746 days, and two animals with marked signs of senility—of 744 and 746 days, killed without fatiguing. In each case 100 cells were examined.

The major conclusions to be drawn from the present work are: 1. There are morphological changes in nerve cells as the result of fatigue carried to exhaustion, consisting primarily in a loss of Nissl material, an increased basophilic property of the nucleus, and an increased average cell size. 2. In senile animals there is also a loss of Nissl substance and an increase in the basophilic properties of the nucelus. The binucleate condition of the Purkinje cell is a phenomenon of senility. The differences between the Purkinje cells of senile and of young animals are far more marked than are those between exhausted and fresh animals of the same age.

Localization in the oculomotor nuclei of the goldfish. Zareh Hadidian, Milton S. Dunn and Roland Walker.

The position of cells innervating the individual eye muscles was studied to see whether there is any pattern in the oculomotor nuclei which might be correlated with the type of eye coördination, which differs from that in animals with binocular vision.

The method was a study of chromatolysis in the oculomotor cell bodies after cutting a peripheral nerve or its muscle, since these operations gave similar results. In normal animals there was a negligible proportion of cells showing advanced degeneration, while in animals with both eyes removed practically all the cells showed chromatolysis. There were no changes in the nuclei studied after removal of the contents of an eyeball.

When the contents of one orbit were removed, thus insuring complete damage to all fibers of III and IV on that side, about half of all the oculomotor and trochlear cells were altered. Chromatolysis in the trochlear nucleus was contralateral, while the degenerate oculomotor cells were about 70 per cent homolateral, the contralateral ones being mostly in the ventromedial portion.

Chromatolysis after damage to the inferior oblique muscle or its nerve was about 80 per cent homolateral, with a slight tendency toward ventromedial localization. For the anterior rectus degeneration was about 60 per cent homolateral with no localization. The inferior rectus showed about 85 per cent homolateral degeneration with definite localization in the dorsolateral nucleus anteriorly, changing gradually toward the ventromedial nucleus posteriorly. The superior rectus had only about 20 per cent homolateral cells, and the contralateral group was localized in the ventromedial nucleus.

A consideration of these results leads to the conclusion that since there is poor definition of cell groups, and scattering of cells for any one muscle throughout the nucleus, any merely anatomical studies of oculomotor localization are inadequate for the understanding of the type of eye coördination in the goldfish.

Some new observations on the secretory activity of neurones. E. Scharrer. (Followed by Demonstration.)

Nerve cells having more or less the appearance of secretory cells have a wide distribution. Among the invertebrates they have been found in annelids, molluscs, crustaceans and insects; among the vertebrates, in the diencephalon of selachians, teleosts, amphibia, reptiles and mammals, including man. In the case of the bony fishes, gland-nerve cells have also been found in the nucleus of the nervus terminalis, in the midbrain and in the caudal region of the spinal cord; the latter being especially well developed in selachians (see Speidel, 1919, 1922). All stages can be found, from typical nerve cells containing only a few granules in the cytoplasma to cells with a spectacular formation and storage of droplets of a colloid-like substance. There are even cases, such as that of the Mediterranean fish *Cristiceps*, where the nerve cells in the so-called "diencephalic gland" are transformed into gland cells lacking any nervous character. A marked nuclear polymorphism is also typical for many gland-nerve cells and pericellular as well as endocellular blood capillaries are often observed in the secretory diencephalic nuclei of vertebrates.

Observations of this kind, even when based on such extensive material, would not suffice to prove the glandular function of the cells in question. It must be demonstrated that there is a functional cycle in the production of the colloid material by the nerve cells in the neuro-secretory regions of the nervous system. This has been done with the diencephalic gland (nucleus praeopticus) of the American toad (*Bufo americanus*). Sections through this gland show all stages,

from the first appearance of fine granules in the cytoplasma of the cells, which stain brilliant orange in Azan preparations, to larger droplets, which finally are extruded and lie as colloid masses among the cells. This cycle can be shown clearly and the identity of those processes in nerve cells with the different stages of secretory activity in gland cells seems sufficiently demonstrated. The physiological meaning of the gland-nerve cells is still unknown and a wide field is opened for future investigation.

Synaptic transmission in the sixth abdominal ganglion of the crayfish. C. Ladd Prosser.

Transmission through the sixth abdominal ganglion of the crayfish was studied by simultaneous recording of impulses entering and leaving the ganglion in response to stimulation of caudal sensory hairs. Flexion of one hair gives rise to one sensory impulse. To set off one efferent unit, however, summation of two to four afferent impulses in different fibers is necessary. No efferent neurone is excited by one incoming impulse. When several efferent units are excited more afferent impulses are required to activate the first efferent unit in a response than to activate later ones. Thus both convergence and overlap play a part in conduction through this ganglion.

Ganglionic delays range from 3 to 30 milliseconds as measured from the time the first sensory impulse enters the ganglion. Most of the fastest units show delays of 5–6 milliseconds, and later ones fall in groups which are multiples of the first. This multimodal distribution of delays is interpreted as indicating the existence of internuncial neurones. A given unit may show fluctuation of 2–3 milliseconds in synaptic delay. The afferent neurones respond to stimuli separated by intervals as short as .01 second, whereas the efferents show recovery times of .05 to .1 second. This synaptic recovery time is longer than that of the fibers, hence no relatively refractory period can be detected in the responses of the individual efferent units.

There are no connections from tactile receptors across the ganglion to contralateral efferent nerves of the sixth segment.

Acetylcholine and eserine have no facilitating action upon the synapse. Eserine is toxic in high concentrations. Nicotine blocks conduction through the synapse. Excess potassium reduces action potentials in the afferent fibers and may block them before affecting the synapse. Adrenaline acts similarly to excess potassium.

It is concluded that those humoral agents which mediate transmission in some mammalian ganglia do not have a similar action in this crayfish ganglion.

JULY 20

Chemical stimulation of the amphibian ectoderm. L. G. Barth.

Further work on the chemical nature of the amphibian organizer indicates that the formation of the neural plate is due to some general stimulus imparted to the cells by a variety of substances. Earlier experiments using cephalin as an inductor showed that cytolysis occurred in the region of the implant. Following this other cytolytic agents such as digitonin, acids and bases were used and plates of neural cells were induced in the presumptive epidermis. Digitonin in concentrations of .5 to .05 % in powdered egg albumen and buffers at pH 3 and 10 have given positive results by implantation into the blastocoel of *Amblystoma mexicanum*.

With regard to the naturally occurring organizer, it was found impossible to extract it completely with fat solvents. In comparing the action of ether-alcohol extracts with protein residues, the protein residue gave better neural tubes. The

ether-alcohol extracts were relatively weak in inducing power. The experiments suggest that the naturally occurring organizer is in the protein residue.

Limb bud transplantation in chick embryos. Viktor Hamburger.

Wing and hind-limb primordia of chick embryos, incubated 2–3 days (25–30 somites) were transplanted to the lateral trunk region or into the coelomic cavity of host embryos of the same stages. In a considerable number of experiments, the anterior-posterior and the dorso-ventral axes were inverted with respect to the axes of the host embryo. The transplants showed complete self-differentiation with respect to form, size and axial pattern.

Transplants located near the spinal cord of the host were supplied by trunk nerves or by nerves branching from the brachial or the lumbo-sacral plexus of the host. The spinal ganglia contributing to the innervation of the transplant reacted always to even small increase in their peripheral fields by hyperplastic growth. In several cases, the number of the motor neurones in the level of the spinal cord which supplied the transplant was likewise found to exceed the number of motor neurones of the normal side, the hyperplasia ranging from 14 per cent to 30 per cent.

Transplants located far ventrally were not innervated by spinal nerves. They showed, nevertheless, normal development and differentiation. The embryonic development of the limbs of the chick is therefore independent of innervation.

Adult organizers and their action in adult tissues. Oscar E. Schotté.

The development of a salamander, Amblystoma punctatum. L. S. Stone (motion picture.)

The development of the common black, yellow spotted salamander, *Amblystoma punctatum*, has been recorded in detail on one motion picture reel showing all the stages from the one-cell egg to the time the larva begins feeding. Rate in development was recorded at various speeds from a few hundred to several hundred times in order to analyse various stages more carefully.

The detailed study in development extends over a period of about four weeks during which time a constant temperature of about 70° Fahrenheit was maintained.

During segmentation one can see clearly the movement of blastomeres, the regional waves of cell division and movement of egg mass due to shifts in the center of gravity. The formation of the blastopore and the development of the neural plate and closure of the neural folds are shown in detail. All the changes in surface development are shown throughout the tail-bud stages from the time at which cilia on the surface of the embryo first come into action to the period when motor activity begins. Occasional local quivering movements in various parts of the embryo are also recorded.

Following these periods in development, dorsal, ventral and lateral views of embryos are shown which carry the growth up to the time the larva begins feeding. The formation of many external features as well as the beginning of peristaltic movements in the intestines are clearly seen.

In appropriate parts of the film are shown clusters of eggs as they are normally laid, circulation in the tail fin, and a view of the larva taking its first meal. The picture begins with a view of a typical habitat and ends with a view of several stages in development from the egg to the adult animal in order to give a comparison of sizes during growth.

JULY 27

The effect of standing on the carbon dioxide content of alveolar air and total ventilation volume. J. K. W. Ferguson and F. A. Hitchcock.

A study of the respiratory exchange during consecutive ten-minute periods of reclining and standing, has revealed in every experiment a decrease of 7–15 per cent in the CO_2 content of alveolar air (confirming Higgins, 1914, Turner, 1927 and Main, 1937). This fall, however, cannot be attributed to overventilation in the usual sense of the word, because: (1) the R.Q. fell during standing in 21 out of 24 experiments, (2) the CO_2 output fell in 12 out of 24 experiments, (3) the total ventilation fell in 9 out of 24 experiments.

When four consecutive periods, with the subject alternately reclining and standing, were studied, it was found that the O_2 consumption and the CO_2 output were greater during the second reclining period than during the preceding standing period. This is interpreted as evidence of oxygen debt and CO_2 retention during standing. It seems probable that this disequilibrium, during standing, between metabolic requirements and the respiratory exchange is due not to inadequate pulmonary ventilation but to inadequate circulation in the dependent parts of the body resulting from the erect posture.

The mechanism of the loss of heat from the human body. Eugene F. DuBois and James D. Hardy.

The three main channels of heat loss are radiation, convection and vaporization. The respiration calorimeter of the Russell Sage Institute of Pathology in New York Hospital measures the vaporization by weighing the moisture that comes from the skin and lungs. It measures radiation plus convection in a stream of cool water that flows through coils in the top of the calorimeter. Radiation is then determined independently by means of a Hardy radiometer pointed in rapid succession at 20 different places on the surface of the body. The total surface is measured and the effective radiating surface calculated as 80 per cent of the total. Convection is then estimated by difference. It has been possible for the first time to separate the factors of radiation and convection in persons exposed to varying atmospheric conditions such as are found indoors.

Two normal men were studied naked at temperatures between 22 and 35°C. Their basal metabolism was uniform throughout this range not rising until a few minutes before the onset of shivering which occurred after exposures of two hours at 22°C. At the higher temperatures there was profuse sweating and practically all of the heat was lost through vaporization. The percentage of heat lost in radiation decreased steadily with rising temperatures. In the neutral zone of 28 to 32°C. convection accounted for 10–15 per cent of the heat loss and radiation 50–60 per cent. Convection was markedly increased by slight movements of the body. With moderate exercise or shivering it accounted for 25–30 per cent of the total loss. An electric fan raised the percentage to 33. Two athletes playing violent squash for 36 minutes showed a 2°C. rise in rectal temperature and about an equal fall in average surface temperature. The total heat lost through radiation increased but little, the percentage fell to 15. Convection during the exercise and recovery period accounted for 5–15 per cent. Vaporization dissipated 70–80 per cent of the heat.

Peripheral inhibition of smooth muscle. Emil Bozler.

The antagonism of vasoconstrictor and vasodilator nerves was studied using perfused frog legs and rabbit's ears and recording the vascular responses by a sensitive flow-meter. Stimulation of the vasodilators of the dorsal roots blocks the action of single volleys of impulses of the vasoconstrictor fibers, whereas re-

petitive stimulation produces a response. Likewise acetyl choline blocks entirely the action of the first few impulses produced by repetitive stimulation. In an attempt to explain these results a difficulty was encountered in the observation that neither vasodilator stimulation nor acetyl choline antagonise the action of adrenaline. The simplest explanation seems to be the assumption that during inhibition the passage of impulses from vasoconstrictor fibers to the muscle is partially blocked, thereby preventing the formation of the adrenaline-like mediator. It is suggested that the increase of polarisation, which has been observed in other cases of inhibition, is the immediate cause of the partial block. The mediator produced by the vasodilator fibers may be the cause of the change of polarisation of the muscle fibers and, therefore, indirectly also of the partial block produced by the activity of these nerve fibers.

The relationship of tissue chloride to blood chloride. William R. Amberson, Thomas P. Nash, Arthur G. Mulder and Dorothy Binns.

A number of previous investigators have found that amphibian muscles, placed in, or perfused with, isotonic sucrose solutions, rapidly lose their chloride almost completely, while retaining almost all of their potassium and phosphate. When such muscles are soaked in solutions of varying chloride content, the tissue chloride varies directly with that of the external fluid. Such observations have led to the conclusion that muscle chloride is extra-cellular, a view reinforced by its low concentration in this tissue.

Other students have recently attempted to extend this concept to other tissues, and to the mammalian body. In studying this literature it occurred to us that it would be useful to know whether the chloride of the mammalian tissues can be diminished when the plasma chloride is lowered. We have found it possible to produce very radical diminutions in the plasma chloride by a modification of the method of total plasmapheresis described by Stanbury, Warweg, and Amberson (*Am. Jour. Physiol.*, 1936, 117, 230). We make up our artificial plasma with sulfates instead of chlorides, adding chloride-free gum acacia, and suspending in this plasma ox red blood corpuscles which have been rendered chloride-free by many washings through sulfate-Ringer-Locke solution.

By long perfusion of the mammalian body we are able to remove most of the chloride before death ensues. In some tissues, such as skeletal muscle, liver and kidney, the tissue chloride falls off in direct proportion to the plasma chloride, as perfusion proceeds. In other tissues the straight line through all the points does not pass through the origin, but shows a y-intercept, suggesting that a portion of the chloride is indiffusible, and presumably intra-cellular. The stomach and spleen have particularly large intercepts of this character.

The central nervous system resists removal of chloride, whereas most of the chloride of peripheral nerve may be washed away. The brain and cord chloride is held either (1) because it is largely intra-cellular, or (2) because the sulfate ion is unable to penetrate into the brain tissue.

Certain tissues, such as tendon and lung, have chloride concentrations so high that it is impossible to explain it all by allotting it to extracellular fluid.

August 2

The use of diatoms from geological excavations at Clovis, New Mexico, as indicators of water conditions. Ruth Patrick.

Mammoth Pit lies between Clovis and Portales in the Staked Plains region of New Mexico. The stratigraphy is as follows: The lowest stratum is coarse gravel devoid of diatoms. Above this is a stratum of speckled sand, in which is a diatom flora which seems to indicate a fresh to brackish water condition by the

dominance of *Anomoeoneis sphaerophora* (Kütz.) Pfitzner, *Amphora ovalis* Kütz., and *Amphora ovalis var. pediculus* Kütz. Horse skeletons are found in this level. The next stratum is a "bluish clay" of about the same constituents as the speckled sand plus considerable carbonized vegetable matter. In the lower part of this stratum, a very rich diatom flora consisting mainly of fresh water species such as *Eunotia arcus* Ehr., *Cymbella affinis* Kütz. *Fragilaria brevistriata* Grun. and *Synedia ulna* (Nitzsch.) Ehr. was laid down. The change in abundance and also in the kind of species from the previous level points to a freshening of the water. This agrees with other geological evidence that this was a period of much higher precipitation than now exists in New Mexico. Mammoth skeletons are most abundant in this level of the stratum. Passing from bottom to top of this stratum the typical fresh water species disappear. The dominant species near the top are the brackish or alkaline water types such as *Epithemia argus* Kütz., *Rhopalodia gibba* (Ehr.) Müll., and *R. gibberula* (Ehr.) Müll. Coincident with the change in diatom species, the mammoth bones disappear and bison skeletons become much more numerous. The top stratum consists of brown dune sand devoid of diatoms. It is in the bottom of this layer that the bison bones disappear. Thus the change in water conditions as indicated by the diatoms seems to coincide with a change in the fauna as shown in this stratigraphy.

Gemmipary in Kalanchoe rotundifolia and other Crassulaceae. Harry N. Stoudt.

The phenomenon of vegetative propagation in Crassulaceae has received much attention from students or morphology and physiology. A comparison of the morphological development of plantlets of this species with other members of the family should aid in understanding more adequately the phenomenon so characteristic of the group.

Yarbrough (1936) reports that apparently mature parenchyma cells in the base of the petiole of *Sedum Stahlii* resume mitotic activity to form plantlets when the parent leaf is removed from the plant.

Stoudt (1934) published an account of vegetative propagation in *Byrnesia Weinbergii*. In this species a plantlet forms at the base of the sessile leaf from a residual meristem that is undifferentiated into plantlet rudiments.

Freeland (1933) discusses this phenomenon in *Bryophyllum crenatum* in which plantlets develop from residual meristems in the notches of the parent leaf. He finds that the amount of differentiation of plantlet rudiments varies. A stem primordium only may be formed by the time the parent leaf is mature.

A nine-millimeter leaf of *Kalanchoe rotundifolia* reveals a meristematic region on the adaxial surface of the petiole. This region is undifferentiated into plantlet rudiments but when the leaf attains its maximum size, three to four centimeters in length, leaf and stem primordia have formed. The bud then remains dormant until the leaf is severed from the parent plant.

Root, stem and leaf primordia are usually visible in the notches of the mature leaves of *Bryophyllum calycinum* according to Naylor (1932) etc. while in *Kalanchoe daigremontiana* and *K. tubiflora* plantlets consisting of root, stem, and leaf primordia are visible macroscopically even before the parent leaf has attained maximum size.

Thus there is a definite sequence into which the various species arrange themselves in respect to the degree to which the meristematic cushion becomes differentiated by the time the parent leaf is mature. Their greatest differences are in the stage of development attained by the meristem, or organ rudiments derived from it, at this time.

Pollen analysis of the air in relation to hay-fever. A. O. Dahl.

It is essential for the successful diagnosis and treatment of hay fever ("pollenosis") that detailed data concerning the concentration of pollen in the air of any species at a given time or locality be made available. Records of the pollen content of the air have been obtained by exposing each day an oil-coated slide out of doors for 24 hours. The pollen grams observed in 25 systematically distributed low-power fields are identified and counted. The approximate number of pollen grains per cubic yard of air can be calculated by use of physical formulae. The pollen grains involved in hay fever in Minnesota vary from approximately 15 to 80 micra in diameter.

Hay fever is a regional problem and atmospheric data from one locality will not apply in a detailed manner to another place. In Minnesota, pollen concentrations of clinical interest occur between late March and early November. For purposes of clinical convenience, the pollens found in the air during the entire season have been placed into 16 groups. Thus, in diagnosis, one scratch-test for each group will test the patient's sensitivity to all pollens present during the entire season. Prefaced by such procedure, successful therapeutic measures can be instituted. (The complete report is to be published in joint authorship with Dr. C. O. Rosendahl and Dr. R. V. Ellis, under whose direction the study has been carried on for the last 5 years at the University of Minnesota).

AUGUST 3

A convenient test of physical agents as producers of dominant lethals. P. W. Whiting.

Dominant lethals occurring in the spermatozoa cause failure of development of zygotes. Since in bisexual reproduction eggs also fail to develop unless fertilized, the two types of male sterility, due to (1) lack of sperm and (2) dominant lethals, cannot be statistically distinguished. In *Habrobracon,* reproducing males by haploid parthenogenesis, matings with males lacking sperm result in as many progeny per day (♂♂) as cultures from unmated females (4.73 ♂♂) or as matings with untreated males (1.21 ♂♂, 3.30 ♀♀). If fathers are X-rayed, males per day are not increased while females per day are decreased (to 2.22 with 2,500 R, 0.62 with 5,000 R, 0.16 with 7,500 R, 0.07 with 10,000 R, and 0 with 20,000, 40,000 or 75,000 R). Since neutrons induce dominant lethals and ultra-short (one-meter) radio waves do not, only the former may be expected to induce recessive lethals and visibles.

Cytological observations on colchicine. Bernard R. Nebel.

The action of the alkaloid colchicine on mitosis was studied in the following material:

Stamen hairs of *Tradescantia,* roots and shoots of *Zea, Vicia, Tomato, Tagetes, Antirrhinum, Trifolium, Papaver, Dianthus, Solanum,* and *Lilium,*—testes of *Podisma,* eggs of *Asterias* and *Arbacia.*

In *Tradescantia* all stages of mitosis are easily seen in life. The cells will continue to divide in salt sugar solutions to which drugs may be added.

Colchicine in concentrations of 5×10^{-4} to 5×10^{-6} molar will stop mitosis during metaphase. In concentrations of 5×10^{-6} to 10^{-6} molar it tends to produce binucleate cells. When blocked in metaphase the plate in *Tradescantia* is characteristically tilted, since a true spindle is not developed. A comparative study of phenyl-, amyl-, propyl-, ethyl urethane and chloral-hydrate showed that these anesthetics within their respective reversible concentration ranges will only occasionally produce binucleate cells. There is no particular evidence of a metaphase

block with the urethanes. In studying the action of colchicine on the developing egg of *Arbacia punctulata* it was necessary to use fixed material to determine the nuclear stage accurately. Colchicine applied 10 minutes after insemination will block the first cleavage metaphase in concentrations above 10^{-4} molar. Between 6.5×10^{-4} and 3.5×10^{-5} molar nuclear divisions of abnormal type proceed while cleavage is impeded. The rhythm of nuclear division may persist so that when controls are in third cleavage metaphase the eggs to which colchicine was added 10 minutes after fertilization will also show approximately 4 groups of chromatin, but the plates are hypoploid, often containing only one to six separate chromosomes, which may partly represent fused units. The micronuclei which form are not far apart from one another and their resting stage is relatively short; with lower concentrations the rhythm of division is not affected in the early cleavages, but the subsequent development of the larvae is markedly stunted even at 10^{-6} molar. Colchicine in increasing concentrations thus first interferes with the normal course of cleavage, then astral rays are prevented from forming, next the spindle is reduced in size and finally obliterated. Meanwhile chromosomes become pycnotic during a prolonged metaphase; they fail to divide orderly; the abnormal division may separate entire chromosomes rather than split halves.

In the plants, in which root and shoot growth was studied (Ruttle) cuttings and seedlings respectively were subjected to colchicine treatments by immersion in aqueous solutions primarily. The active concentration ranges were found to be the same as in *Arbacia* and *Tradescantia*. All genera showed marked reactions, the tomatoes being the least sensitive. Where plant meristems were treated, the resulting tissues showed markedly irregular growth—incised and crumpled leaves as well as chlorophyll defects. Cytological investigation showed necrotic cell lineages and multinucleate cells in varying degrees. The drug is being studied further as an agent which may induce mutations and polyploidy.

In coöperation with other investigators, some physiological effects of colchicine were investigated. Respiration was tested on *Arbacia* eggs with colchicine 1.8×10^{-4} to 7.5×10^{-4}. Respiration did not vary from the control in any of these concentrations (Tyler).

Pectinmethoxylase (pectase) from tomato juice showed inhibition which is of doubtful significance since with higher concentrations of colchicine a precipitation was observed in the reaction mixture (Kertesz).

The nitroprussiate reaction for $S-H \rightleftharpoons S-S$ groups gives a color reaction in stamen hair cells of *Tradescantia*, the color being located in the chromonemata and in certain plasmatic granules. Cells under colchicine gave the same reaction (Medes).

No significant influence was observed on the action of carbonic anhydrase from blood.

No inhibition occurred of the reduction of methylene-blue by yeast or blood with and without admission of air.

No sensitization of *Arbacia* eggs to X-rays was obtained by the addition of colchicine, when nuclear abnormalities were used as a criterion.

Demonstration of vital staining preserved in paraffin sections of lamprey embryos (Bismark Brown method). R. Weissenberg.

In 1929 I recommended Bismark-brown for localized vital staining of the egg of lamprey because it is very easy to preserve these stained areas for paraffin sections.

The quick-working method which I am using now in my studies of localization on lamprey egg is a very simple one. It is based on the surprising fact that Bismark-brown as a vital dye is alcohol-proof without further treatment in contrast to Nile-blue sulfate.

I fix the embryos in a mixture of 1.5 parts of absolute alcohol and 0.5 part of acetic acid for ten minutes, wash in absolute alcohol for a few minutes, and transfer them directly into cedar oil.

The stained areas are very well preserved by this simple method and the eggs can remain in the cedar oil for years without any loss of the dye. They can be studied in the cleared condition in the cedar oil as total preparations, or they can be imbedded in paraffin at any time and cut with excellent preservation of the vital stain within the sections.

The slides demonstrated are balsam preparations seven years old and still give a true representation of the stained areas of the living organism. The preservation obtained by this method is complete also in the finer localization of the staining. Carriers of the vital staining in the egg of lamprey are chiefly the yolk granules because here, in contrast to most amphibian eggs, pigment granules are missing in the earlier stages of the embryo.

Microfilm on some experiments on isolated amphibian germinal vesicles. William R. Duryee.

The film shows colloidal changes in the frog ovocyte nucleoplasm, nucleoli, and chromosomes brought about by relatively slight changes in the Na, K, Ca chloride concentrations of the medium. H^+ ions reverse the normal negative charge on the nuclear components to positive. When this change is gradual enough, as with 0.003 N HCl, a dark converging "ring" forms from flocculating particles in the approximate pH region of 4.0 to 5.0.

Ca, Mg, Cu, Hg and basic dyes behave similarly to H^+ ions in causing a phase separation and an appearance of chromosomes from a previously transparent nucleus. On the other hand, K and Na and especially OH^- tend to disperse the nuclear colloids, thus stretching and separating the chromosome pairs, and at the same time making the nucleus transparent. Within narrow limits these changes are reversible.

In *Triturus pyrrhogaster*, the "Binnenkorper," or first maturation spindle anlagen situated at the center of the germinal vesicle, can be made to swell and separate the chromosomes radially, but not in typical bipolar directions. *Rana fusca* appears unique in having a differentiated coagulable capsule around the chromosomes, which may be important in forming the denser portions of the spindle. In *R. pipiens* the contraction of this material under the influence of calcium is less striking.

Similar changes, including phase separation and violent contraction of the chromosomes, occur when acid fixatives are added or when the nuclei are exposed to their disintegrating cytoplasm. Hence this latter effect may be termed *autofixation*. Prominent differentiated areas or sac-like projections of the nuclear membrane reversibly swell and shrink in bases and acids respectively. Such structures are obliterated by fixatives. It is concluded that in the forms studied Darlington's assertion as to the absence of a nuclear membrane must be modified. With merely fine forceps and pipette it is easily possible to isolate various components of these giant nuclei (0.8 mm. diameter),— e.g. nuclear membrane, spindle anlagen, nucleoli, and chromosome pairs (100–200μ)—during the fall, winter and early spring months of the year.

Cortical cytolysis of the echinoderm egg. Robert Chambers. (Motion picture.)

Motion pictures were taken of those experiments designed to demonstrate the physical properties of the cellular cortex particularly in marine ova. Nearly all experimentally induced effects on living cells are extremely transitory. A later study of the motion picture record permits of a more careful and complete analysis

of these phenomena. For example, the film may be slowed down, stopped at any point or reversed at will. The film presented at the seminar is a compilation of many of these experiments.

Included in the film is a scene showing the spontaneous coalescence of a denuded *Arbacia* egg with an oil drop. From such a record it is possible to measure with considerable precision the diameter of the oil drop at the instant of coalescence. These data are essential if the coalescence phenomena are to be treated quantitatively, yet it is practically impossible to obtain the oil drop sizes in any other way.

Another scene shows the natural elevation of the cellular surface and the reaction of this surface to mechanical injury. Over-insemination of an immature *Arbacia* egg results in the formation of many large insemination cones. Gentle manipulation with a microneedle will cause these cones to run together, thus bringing about an elevation of a continuous film which is separated from the granular cytoplasm by a liquid space. If this elevated film is ruptured at one spot with a microneedle, the entire film will rapidly disintegrate. The underlying cytoplasm becomes exposed and it quickly becomes converted into an irreversible coagulum.

The following phenomena, *inter alia*, are also demonstrated: cytolysis of starfish eggs in hypotonic sea water, effects of tearing eggs in pure salt solutions isotonic with sea water and the shrinking of the cortex of one blastomere following the puncture of the other.

The photography was done by Mr. C. G. Grand.

August 10

Some aspects of normal and regulative development in the colonial ciliate, Zoöthamnium alternans. F. M. Summers.

A remarkable number of studies on metazoan "organizers" have already demonstrated the importance of extrinsic factors for determination in specific parts. It was felt that additional information about these factors could be gained by applying operative techniques to an animal type in which, presumably, the relationships of parts have not attained so great a degree of complexity. *Zoöthamnium alternans* is a protozoan colony whose cells collectively possess in some degree many of the attributes of an individual organism. It is admirably adapted to this type of work for many reasons, particularly by virtue of the precision with which the characteristic colonial pattern develops.

One of the most important consequences of this study of more than 200 normal and operated colonies is the demonstration of qualitatively different physiological relations between cells at different locations on the colonial framework. Under normal conditions a specific pattern unfolds. When the cell at the apex of the frond-like colony is cut away some cell of a lower order, one whose complete developmental potentialities are never otherwise expressed, assumes the dominant generative functions and the normal colonial pattern perseveres in the parts regenerated by it. These results are intelligible in terms of what Child (1929) calls physiological correlation: the relations of dominance and subordination between parts. Apical control appears to be continuous and quantitative.

In this organism the transformation of the apical cell into an ex-conjugant initiates a developmental phase which furnishes another clue to the general nature of apical control. About four days after the union of gamonts, even before ex-conjugant generations are produced, the normal developmental relations are upset in an unusual way. The first three or four branches below the conjugant level begin to develop out of all proportion to the normal expectations. Each branch develops almost as an individual colony. Its cells divide precociously, forming secondary and even tertiary branch strains. The greatest effect obtains on the branch nearest the conjugant and diminishes basally as a gradient; the basal branches are apparently unaffected.

Under varying physiological conditions in the apical cell the coördinating influences exerted upon the mitotic activity of neighboring cells may be inhibitory (as shown by the regulative response after the apical cell is removed) or excitatory (when the apical cell is transformed into an ex-conjugant). The precocious development does not occur when the apical cell is present *or* when it is dissected away; it appears to be effected by some new quality in the coördinating mechanism arising in consequence of conjugation activities in one particular cell—the apical cell. These results invite the conclusion that the integrative factors in a colony of *Zoothamnium* are qualitative and discontinuous.

Morphology, behavior and reproduction in Type A and Type B of Chaos chaos Linnaeus, the giant multi-nucleate amoeba of Roesel. A. A. Schaeffer.

Chaos chaos Linnaeus 1767, the first amoeba to be discovered, was described by Roesel von Rosenhof, a painter of miniatures, in 1755, in Germany. It has been seen 5 times since then: in 1900 by H. V. Wilson, North Carolina; in 1902 by E. Penard, Switzerland; in 1916 by W. A. Kepner in Virginia and by A. A. Schaeffer in Tennessee; in 1936 by A. A. Schaeffer in New Jersey.

The general morphology and behavior of this amoeba are so much like those of the common laboratory amoeba, *Chaos diffluens* Müller, 1786, that there is strong probability that both amoebas belong to one and the same species. Conclusive evidence of such relationship is, however, still lacking and therefore, until such evidence is found, the two taxonomically specific names, *C. chaos* and *C. diffluens*, will be used to avoid confusion.

C. chaos falls into at least two types which are distinct in some morphological details and also in antigenic reactions. Type A has discoid nuclei and divides usually while freely rolling around on the substrate. Type B has broadly ellipsoid nuclei, and divides while fastened to the substrate. The nuclei of type A are larger than those of type B. With the collaboration of Dr. J. A. Harrison, preliminary antigenic tests were made in which it was found that there is a marked difference between the two types, type B standing closer to *diffluens* than to type A.

Three of the most striking differences between *chaos* and *diffluens* are: in size, *chaos* being from 50 to 500 times as large as *diffluens;* in number of nuclei, *chaos* being multinucleate (up to 1,000 or more); in reproduction, *chaos* dividing at any single division, into 2, 3, 4, 5 or 6 daughters, with a strikingly marked mode at 3 daughters.

Pieces cut from *chaos* grow up to full size, whether the piece contains one or more nuclei.

Both types give off, when crushed, a strong cucumber-like odor which can readily be detected when only one amoeba is crushed on a slide. In *diffluens* this odor is also present but to a much smaller degree.

Observations upon the chemical composition and the metabolism of a larval parasitic nematode. Theodor von Brand.

The experiments were performed with an immature *Eustrongylides* from *Fundulus heteroclitus.* The red color of the worms is due to the presence of hemoglobin in the body fluid. With its low fat and high glycogen content the general chemical composition resembles that of the adult *Ascaris.* The worms consume per unit weight much less glycogen than *Ascaris,* if kept in saline at 37° C. under aerobic conditions. They are able to keep their glycogen level high, even if their hosts starve for a long time and lose during this starvation period more than half of their polysaccharide stores.

Observations and experiments on sex change in the adult American oyster, Ostrea virginica. Paul S. Galtsoff.

Sex of oysters was determined by inducing ovulation or ejaculation by increased temperature and chemical stimulation. During the summer of 1936 individual spawning records of 203 adult oysters were obtained and the discharged products microscopically examined. Oysters were measured, carefully marked by numbers engraved on the shell of each, and transferred to Milford, Connecticut, to be kept in tidal tanks through the winter. The same procedure was repeated during the summer of 1937.

Results showed that 9.7 per cent of the oysters reversed their sex; the percentage of reversals being higher among females (13.1 per cent) than among males (8.0 per cent). Mortality during the year was only 7.04 per cent and failures to react, due to lack of gonad development, was only 1.07 per cent, indicating healthy conditions under which oysters were kept.

In the sex-reversed males the physiological set-up of the organism changes with the sex; typical female reaction, characterized by the rhythmicity of contractions of adductor and maintenance of a constant tonus level, develops in the former male. In several instances development of this reaction lagged and the newly-formed female retained physiological characteristics of the male, i.e., eggs were discharged through the cloaca instead of through the gills and rhythmical contractions of adductor were absent. All sex-reversed females reacted as true males.

It is concluded that female reaction developed as a secondary adaptation providing mechanism for dispersing eggs throughout the water. From the simultaneous occurrence of sex reversal in the oyster population the conclusion is made that Orton's theory of metabolism change (protein to carbohydrate) as the sex-determining factor in *O. edulis* is not applicable to *O. virginica.*

AUGUST 17

A sea water buffer for marine eggs. Albert Tyler and Norman H. Horowitz. (This paper has already appeared in full in *Science,* July 23, vol. 86, pp. 85–86.)

The effect of CO_2 upon the oxygen capacity of the blood of some fresh-water fish. Edgar C. Black and Laurence Irving.

Conditions of respiration for fish differ from the respiratory conditions for mammals. The respiration of fresh-water fish must proceed in a medium in which the pressure of oxygen is always less and the pressure of carbon dioxide usually greater than in atmospheric air. At different levels in the water, pressures of gases are altered by the temperature changes. Types of blood which are suitable for the transport of CO_2 and oxygen under one set of conditions might be quite unsuitable under another set. The characteristics of the blood of the carp (*Cyprinus carpio L.*) and the common sucker (*Catastomus commersonii*) show examples of two types of blood, each suited for a different and limited range of pressures of oxygen and CO_2.

Oxygen dissociation curves obtained from those two species are not as sigmoid as are those for mammalian blood. The presence of 5 or more mm. CO_2 (for the carp 10 or more) prevents the complete saturation of whole blood, even at high partial pressures of oxygen. This effect of CO_2 is quite different from the familiar effect of CO_2 upon mammalian blood. In the presence of relatively high pressures of CO_2 the blood of the carp is suitable for the transport of small quantities of oxygen, while the blood of the sucker would be quite useless.

At very low pressures of CO_2 and high pressures of oxygen in the water the blood of the sucker can serve to transport much more oxygen than that of the carp.

Hemolysis by the addition of saponin to the blood of the sucker, carp and bowfin (*Amia calva*) abolished the effect of CO_2 upon the oxygen capacity at high pressures of oxygen (150 mm.). The CO_2 effect is in part at least dependent upon the integrity of the corpuscles.

Oxidative mechanisms in the resting and fertilized sea-urchin egg. Irvin M. Korr.

Since cyanide inhibits certain iron-containing systems, and since pyocyanine, a bacterial respiratory pigment, functions as a "hydrogen-carrier," it was possible to vary the relative proportions of respiration going through simple, non-ferrous carrier and that going through the cytochrome-indophenol oxidase system; by adding KCN and pyocyanine, separately and in various combinations, to sea urchin eggs. The respiratory rates of the untreated fertilized and unfertilized sea urchin eggs, and those in which the mechanisms had been altered as above, were measured at different temperatures. These experiments were designd (1) to give the temperature relations of the two types of respiration, (2) to throw further light upon the factors determining temperature coefficients of cellular respiration and (3) upon the change in oxidative rate and mechanism that occurs upon fertilization of the sea urchin egg.

It was found, first (in partial confirmation of Rubenstein and Gerard, 1934), that the temperature coefficient of unfertilized eggs was much higher than that of the fertilized egg. The effectiveness of KCN was found to increase with temperature.

Increasing the respiration of the fertilized egg with added hydrogen-carrier did not appreciably change its temperature coefficient. Fertilized eggs, in which the iron system had been maximally inhibited and the respiration restored to or above normal with pyocyanine, also had the same temperature coefficients as the untreated fertilized eggs.

In the unfertilized egg, whose respiration is cyanide-stable, the addition of carrier not only increases the rate of respiration, but also, above a certain concentration, lowers the temperature coefficient. A concentration of pyocyanine which increases the respiratory rate to that of the fertilized egg also lowers the coefficient to that of the fertilized egg.

The results, in conjunction with older work, lead to the conclusions that (1) respiration through a simple non-ferrous carrier and that through the cytochrome system do not, per se, have different temperature coefficients, that (2) these H-transfer mechanisms are the rate-controlling link in the respiration of the fertilized and unfertilized egg. (3) Temperature coefficients are largely determined by the ratio of the rate at which H-atoms are transferred, from substrate to oxygen, to the maximum rate at which they can be produced by the substrate-dehydrogenase systems. The more nearly the H-transfer rate approaches the maximum H-production rate, the lower the temperature coefficient, and vice versa—within the limits set by the fertilized and unfertilized egg.

Methods for the study of rapid chemical reactions and their application to the kinetics of enzyme-substrate and enzyme-inhibitor compound formation. Kurt G. Stern and Delafield DuBois.

The observation of spectroscopically defined enzyme-substrate and enzyme-inhibitor compounds, made in the course of recent studies on catalase and peroxidase, offers an experimental approach to the detailed analysis of the mechanism of

action of these enzymes. A photoelectric method for the recording of such processes and some preliminary results were reported last year (K. G. Stern and D. DuBois, *J. Biol. Chem., 116,* 575 (1936)). This method has since then been improved by replacing the single photoelectric cell by a differential photometer containing two photocells. The technique of mixing by injecting one reactant into the solution of the other with a spring gun has been retained. The new apparatus is quite insensitive to any but color changes. In addition, a simple spectrographic method has been developed which permits the recording of fast reactions without the use of photocells or electric instruments. This is achieved by replacing the plate holder of a spectrograph by a falling plate camera. While the plate is falling, the trigger of the spring gun is released and mixing is complete within 8 to 27 milliseconds. The time is recorded by a rotating time marker or a Neon tube flash circuit. With this method the reaction of catalase and methemoglobin with hydrogen peroxide, ethyl hydrogen peroxide, hydrocyanic acid, and hydrofluoric acid has been studied. The reaction rate is greatly dependent on the concentration of the reactants and on their ratio, as would be expected from a bimolecular process. The reactions studied appear to be slower than the reactions of hemoglobin or hemocyanin with oxygen or carbon monoxide which have been measured by Hartridge, Roughton, and G. A. Millikan with the flow method. Only when a considerable excess of substrate or inhibitor over the catalysts is employed does the rate of the reactions studied by the present authors approach the length of the mixing time which is, of course, the limiting factor in such experiments. Inasmuch as the rate of fall of the photographic plate may be varied within wide limits (0.3 to 29 cm. per second), a wide range of reaction rates may be studied. The use of supersensitive panchromatic plates permits the recording of changes of light absorption in the red and green region of the spectrum at rates of fall of the plate corresponding to an exposure time of less than 0.002 second for an individual spectrum. The continuous strip of spectra recorded on the plates corresponds to 350 individual spectra.

AUGUST 24

Mechanism of cellular death by freezing. B. Luyet. (The essentials of the paper were published in the August issue of *Biodynamica.*)

Binding and penetration of bivalent cations in Elodea cells. Daniel Mazia.

The fact that *Elodea* cells contain soluble oxalates in their vacuoles makes it possible to study by a direct method the binding of Ca, Sr, and Ba ions. By subjecting the cells to strong electric currents or to ultraviolet radiation or to plasmolysis and deplasmolysis, one can set free the bound Ca in their protoplasm, which can then be observed as a precipitate of distinct calcium oxalate crystals in the vacuole. The Ca must come from the protoplasm, for the cells are kept immersed in distilled water or non-electrolyte solution.

That the Ca actually is bound in living cells is indicated by the fact that it cannot be washed out by prolonged immersion (up to 14 days) in distilled water, although it can easily be removed by a few minutes of washing in a citrate solution and replaced then by a few seconds of immersion in a 0.01 M $CaCl_2$ solution. Further studies on leaves from which the Ca has been removed by citrate show that it can be rebound to a maximum level from Ca solutions as dilute as 5×10^{-4} M, the time required increasing with the dilution. This binding is influenced by Na and also by K ions, so that when the Na/Ca ratio is greater than about 100, the binding of Ca is largely prevented.

It is possible to substitute Sr and Ba in the place of Ca in the protoplasm. They are bound in the same way as Ca. Cells in which they have been substituted

for Ca seem to function normally, but when subjected to agents regularly causing the release of Ca show characteristic crystals of strontium oxalate or barium oxalate in their vacuoles.

The Sr ion penetrates the protoplasmic layer easily, even when there is no concentration difference between the Sr inside and outside or even a higher concentration inside. It can be demonstrated that this transport requires first the binding of the Sr in the protoplasm. If Na or K is added to an Sr solution in sufficient concentration to prevent the binding of Sr in the protoplasm, the penetration of Sr does not occur, whereas, in the control, a pure $SrCl_2$ solution of the same concentration, the transport does occur, and crystals of strontium oxalate can be seen in the vacuole.

Factors governing cellular responses to nitro and halo phenols. G. H. A. Clowes, A. K. Keltch and M. E. Krahl.

A recent comparison of respiratory stimulating and cell division blocking effects of three closely related compounds having pK values ranging from 4.1 to 4.5, mononitrocarvacrol, dinitrocarvacrol and dinitrothymol, shows that the first and third substances exert little or no effect on respiration but block division at the extraordinary dilution of 2×10^{-6} M. The second increases respiration almost four-fold to a peak at 10^{-5} M, at which concentration division is blocked. A fourth structurally related substance, 2,4-dinitro-*o*-isopropyl phenol, having a pK of 3.0, produces a moderate effect, giving a respiratory peak and cell division block at 10^{-3} M. These results lend further support to the conclusion previously reached that, while the substituted phenol anion undoubtedly exerts a significant effect inside the cell, it is quite impossible, from a knowledge of only the dissociation constants of such substituted phenols, to predict with certainty the range of concentrations, if any, in which a given compound will affect either cell respiration or division or both.

From experiments conducted in 1935 and confirmed in 1936, in which varying numbers of eggs were used in a constant volume of sea water medium, certain substituted phenols were observed, contrary to general experience with anesthetics, to block division at greater dilution when larger numbers of eggs were employed and vice versa. This was believed to be attributable to a rise in intracellular acidity due to CO_2. At last year's meeting it was demonstrated that with varying CO_2 tensions, incapable in themselves of blocking division, the division-blocking effect of substituted phenols was greatly enhanced.

In an attempt to evaluate the relative rôle of undissociated substituted phenol molecule and anion, the dissociation constants of some thirty substituted phenols have been determined during the past winter and used in analyzing the respiratory stimulating and cell division blocking effects obtained with the compounds in question at fixed exterior and varying interior acidities. These will be reported in the following paper.

On the assumption that the substituted phenols penetrate the living cell only as undissociated molecules, for a given total concentration of the phenol, the intracellular concentration of phenol molecule and phenol anion in an intracellular aqueous phase may be calculated for any levels of extracellular and intracellular acidity.

The possible rôle of acidic dissociation in the physiological effects produced by nitro and halo phenols. M. E. Krahl, G. H. A. Clowes and A. K. Keltch.

From experiments performed during the summer of 1935, using a constant extracellular pH of 7.5 and a constant intracellular pH approximating the normal

6.8, the intracellular concentrations of phenol molecule and phenol anion necessary to give 50 per cent reversible inhibition of division and approximately optimum respiratory effect (where this effect was present) were calculated for 30 substituted phenols. The necessary concentration of phenol molecule was found to vary from 3×10^{-10} M for 2,4-dinitro-*o*-cyclohexyl phenol to 3×10^{-4} M for *m*-nitrophenol. The necessary concentration of phenol anions likewise varied over a wide range, from 1.0×10^{-1} M for 2,4-dinitro-*o*-cyclohexyl phenol to 2×10^{-4} M for 2,4-dinitro-*o*-isopropyl phenol. In confirmation of our previous work and contrary to the results of Tyler and Horowitz (*Proc. Nat. Acad. Sci.*, 23: 369, 1937), picric acid and *o*-nitrophenol, when free of impurities, produce no reversible stimulation of respiration or reversible cell division block. It is clear that no final conclusion regarding the precise rôles played by the phenol anion and undissociated phenol molecule can be reached from such experiments, and that there is some as yet undetermined factor involved. The conclusions of Tyler and Horowitz, who used a limited number of compounds which happened to fall, for the most part, in the middle of the above series, are therefore not justified by the evidence at present available.

With a constant extracellular pH and a decreasing intracellular pH, the ratio of intracellular anion to intracellular substituted phenol molecule decreases, while the concentration of phenol molecule is independent of intracellular pH and dependent only on the total extracellular concentration of phenol and the extracellular pH. In experiments on fertilized eggs of *Arbacia punctulata*, it was found, as anticipated, that at constant extracellular pH of 6.7, the concentrations of 2,4-dinitrophenol, 4,6-dinitro-*o*-cresol, 2,4,5-trichlorophenol, 2,4-dichlorophenol, and *m*-nitrophenol necessary for 50 per cent division block were not affected by decrease in the intracellular pH, while the optimum levels of respiratory stimulation were decreased with decreasing intracellular pH, this decrease in respiratory optimum being largest with 2,4-dinitrophenol and 4,6-dinitro-*o*-cresol which have pK values of 4.1 and 4.4 respectively, and small or negligible with 2,4-dichlorophenol and *m*-nitrophenol which have pK values of 7.7 and 8.3 respectively.

Depolarization of muscle and nerve membranes by organic substances. Rudolf Höber and Bernard R. Nebel.

It is fairly generally accepted that some surface film of muscle and nerve fibers is the seat of a polarized state resulting from the high content of the interior of the fibers in free K ions and from the selective permeability of this film to cations. Furthermore, it is believed that the negative electric wave sweeping along the fibers after excitation is indicative of a local and reversible propagated depolarization due to an increase of ion permeability. This increase would be the result of an electro-chemical reaction, which involves a structural alteration of the surface film. Since the excitation process has been shown to be connected with an increased metabolic activity of the fibers, it seemed worthwhile to study whether organic compounds either identical or more or less related to normal constituents of the fibers would bring about depolarization.

Experiments were performed on sartorius muscles and sciatic nerves of the frog, complemented in coöperation with Dr. M. Andersh, by studying nerves of the spider crab. Injury potentials were measured, following the usual procedure.

The experimental result is this, that not only certain organic cations, comparable to the normally penetrating K ions, but also certain organic anions and non-electrolytes are enabled to depolarize the surface membrane, as disclosed by the arising electronegativity. The active cations concerned are those of higher dialkylamines and of alkaloids, the anions those of higher fatty acids and bile acids, the non-electrolytes anesthetics and saponin-like compounds. All these

substances are likewise significant by their cytolytic power, which is associated with a polar structure of their molecule, with surface activity and lipoid-solubility. Lytic effects frequently appear to be irreversible. But under certain conditions, e.g., with the fatty acids by shifting the pH from a more acid to a more alkaline reaction, the depolarized state can be returned to the normal polarization.

These statements tempt one to raise the question whether reversible cytolysis may play a rôle in producing the traveling negativity, the propagated reversible disturbance of the surface film of the excited fibers. Support may be lent to such an assumption by the facts that the phosphatides, characteristic constituents of the plasma membranes, are containing surface-active higher fatty acids and that electric currents, in passing artificial membranes, comparable to the action currents accompanying excitation, have been demonstrated to alter the ion concentrations, particularly the H ion concentrations in the electrolyte solutions bordering the membranes. Under such conditions, in the membranes various physico-chemical or chemical events could be released.

General Scientific Meeting

August 26

On some conditions determining sub-cooling in plant tissues. B. J. Luyet and E. L. Hodapp.

Organisms found in frozen water or on the frozen ground are said to be sometimes killed by a slight concussion incapable otherwise of harming them. This would be attributable to the sudden freezing of the sub-cooled tissues under the action of the concussion. In the present experiments we studied the conditions determining sub-cooling and freezing after sub-cooling, in the potato tuber. Sub-cooling was found to occur with about the same frequency in living and in dead tissue. The temperature to which the material was heated before being cooled had an evident influence on preventing sub-cooling. Freezing of the sub-cooled tissues can definitely be induced by concussion, although the relatively high percentage of inefficient shocks indicates that some unknown factors, uninfluenced by concussion, hold the system in the sub-cooled condition.

On the double freezing point of some living tissues. B. J. Luyet and Sister P. M. Gehenio.

Some plant tissues have been found to present sometimes two freezing points, one a few tenths of a degree below zero and the other about a degree lower. In the present work we investigated the conditions in which one obtains the double freezing-point in the potato tuber. From a large number of determinations it results that it is a character of living tissue and that a congelation of the material at the first freezing-point does not kill it, while at the second it does. The presence of the double plateau in the freezing curve, and its shape, have been studied in terms of the cooling velocity, of the occurrence of sub-cooling, of the size of the piece of tissue, of its degree of desiccation or of imbibition, and of the type of thermometric device used. The possible factors responsible for the double freezing point are discussed.

Transverse electric impedance of the squid giant axon. H. J. Curtis and K. S. Cole.

The transverse electric impedance of the giant axon of the lateral mantle nerve of the squid has been measured by means of a Wheatstone bridge over a frequency range from 200 to 2,500,000 cycles per second. The bridge current was

at all times kept well below that necessary to stimulate, and excitability was tested at the end of each run.

The phase angles measured for this axon ranged from 70° to 85° which indicates that the membrane impedance is of the polarization type. These phase angles are considerably higher than those found for nerve bundles from the same animal, so it seems very likely that part of the low phase angles found for nerve bundles may be due to a statistical distribution of fiber diameters and membrane capacities. In several cases, impedance runs were taken both before and immediately after the fiber lost excitability, and none of the impedance characteristics of the axon changed when this occurred. Some time later, however, the membrane impedance dropped to zero which indicated the death of the cell. Membrane capacities found for this axon average 0.42 μf/cm.^{2} at 100,000 cycles, which is in good agreement with the values previously found for nerve bundles.

Electric impedance of suspensions of unfertilized and fertilized Arbacia eggs. K. S. Cole and J. M. Spencer.

The alternating current resistance and capacity of suspensions of *Arbacia* eggs in sea water, measured at frequencies from one thousand to ten million cycles per second, give average membrane capacities, with 90° phase angles, of 0.84 μf/cm.^{2} for the unfertilized, and 3.5 μf/cm.^{2} for the fertilized eggs. Some slightly lower phase angles were probably indications of abnormalities. The previously reported additional capacity element in the fertilized eggs disappeared as the season progressed. It was found that the plasma membrane enclosed volume averaged 1.7 per cent less than the non-conducting volume for the unfertilized egg and 2.5 per cent less for the fertilized egg, while the fertilization membrane enclosed volume averaged 32 per cent greater than the non-conducting volume. Thus the plasma membrane lies very close to the non-conducting membrane in both the unfertilized and fertilized egg, and the fertilization membrane is practically perfectly conducting. It is then probable that, on fertilization, the plasma membrane capacity increases to some four times its unfertilized value.

Electric impedance of single Arbacia eggs. K. S. Cole and H. J. Curtis.

The end of a two or three-mm. thin-walled glass tube is heated until it closes down to a short capillary about 50 μ in diameter. The tube is partially immersed in sea water, and when an egg, dropped in the upper open end, settles to the top of the capillary, the water level in the tube is raised until the egg is pushed into the middle of the capillary. Impedance measurements are then made between electrodes placed in the tube and in the outside solution. The low frequency resistance of a 48 μ tube rose from 24,000 ohms, when filled with sea water, to 840,000 ohms with an unfertilized egg in place. This increase might be due to a membrane resistance of 20 ohm cm.^{2} but this value is no more than a lower limit since a layer of sea water 0.25 μ thick between the egg and the glass would produce the same result. The low frequency resistance for a fertilized egg was equivalent to a 4.2 μ space, which is larger than the membrane elevation. The observed low frequency capacities and the higher frequency data give average membrane capacities of 0.8 μf/cm.^{2} for the unfertilized, and 2.8 μf/cm.^{2} for the fertilized eggs. These results are in agreement with these obtained from suspensions and the technique may be used for several problems which are not otherwise possible.

The effect of NaCl on potentials in Nitella. Samuel E. Hill.

The normal action current in *Nitella* requires about 15 seconds for completion, including recovery. After the cells have soaked for 30 minutes or more

in 0.01 M NaCl the action currents may become very brief, lasting not more than 1 or 2 seconds. The form of the action curve changes, showing 1 peak instead of 2, and the amplitude is usually less.

After 24 hours in 0.01 M NaCl the action curve tends to become normal again.

The cells show no signs of injury after 24 hours or more in 0.01 M NaCl.

The coalescence of a plant cell with oil drops. M. J. Kopac.

The young aplanospores of *Valonia ventricosa* are essentially naked protoplasts and coalescence with oil drops readily occurs. As the aplanospores become older, the tendency to coalesce with oil drops becomes decreased. Coalescence with oils of a high interfacial tension against sea water (paraffin oil, tension *ca.* 40 dynes^{1} cm.$^{-1}$) is inhibited in aplanospores over 3½ hours old. This decreasing tendency to coalesce with oil drops is believed to signify the building up of extraneous coats by the protoplast. Ultimately the aplanospores become coated with a cellulose wall.

In aplanospores about 1½ hours old, coalescence with low tension oils (oleic acid in paraffin oil, tension *ca.* 3 dynes^{1} cm.$^{-1}$) occurs rarely. A small drop of this oil may be placed in contact with an aplanospore without coalescence occurring. If a few seconds later a small drop of a higher tension oil (oleic acid, tension *ca.* 10 dynes^{1} cm.$^{-1}$) is applied to the opposite side, coalescence between it and the aplanospore immediately takes place. From ½ second to several minutes later, the first drop snaps into the aplanospore. In these young aplanospores no cellulose cell wall has been formed. The inhibition of coalescence with a low tension oil may be due to the preliminary solidification at the cell surface prior to the formation of a cellulose wall. Coalescence with oleic acid apparently induces a peripheral disorganization at the cell surface which then permits the protoplast to coalesce with a low tension oil. This disorganization in the case of the aplanospore may actually be a disintegrative action at the cell surface. Additional evidence for this point is shown by the release of chloroplasts from the protoplast following coalescence with the two drops. These investigations were started at the Tortugas Laboratory this summer and are being continued at the Marine Biological Laboratory.

The influence of length, tension, and tone upon the birefringence of smooth muscles (*Phascolosoma and Thyone*). Ernst Fischer.

The retractor muscles of *Phascolosoma* and *Thyone* respond to direct stimulation with a twitch-like contraction. After indirect stimulation involving the ganglion the quick contraction of both muscles is followed by a sustained tonic contraction. Besides this "contractile tone" both muscles show marked "viscosoidal tone." A muscle stretched by a load is constantly lengthening, and when the muscle is released later on, it shortens only to a small extent. By alternatingly loading and releasing a muscle with increasing weights, the birefringence of the muscle can be measured for the same muscle length under no tension and under a well-determined tension. When no tension is exerted, the birefringence of the muscle is about proportional to the square root of the length, as found by Bozler for a smooth snail muscle under comparable conditions. For the same muscle length under tension the birefringence is higher, the increase being proportional to the tension present,—a true "photoelastic effect." In consequence, when a muscle is stretched quickly, the birefringence increases at a steadily growing rate until suddenly the birefringence diminishes and the muscle tears through.

Under isometric conditions the tonic sustained contraction increases the double refraction under all conditions, while for the twitch-like contraction, as shown

previously, the direction of the birefringence change is dependent on length and tension. This indicates that "normal contraction" and "tonic contraction" are fundamentally different processes. For both muscles investigated the same results were obtained with the only difference that in *Thyone* the birefringence is merely 60 per cent of that in *Phascolosoma*.

The mechanism of salt penetration in Amoeba—some micromanipulative data. Samuel A. Corson.

Addition of inorganic acids (to a pH of 4.5 or lower) to K or Na salt solutions in which the amoebae were immersed prevented the decrease in protoplasmic viscosity and the cessation of movement which occurred when *Ameba proteus* (*Chaos diffluens*) was immersed in neutral or alkaline solutions of these salts. The same acids also prevented the marked increase in protoplasmic viscosity produced by immersion in neutral or alkaline Ca salt solutions. Utilizing the Chambers micromanipulator and a new method which permits quantitative injections (the method consists essentially of enclosing the solution between a layer of heavy and one of light oil and measuring the quantity of solution drawn into the pipette by means of a calibrated ocular micrometer) it was shown that while KCl injections produced the same effects as in the immersion experiments (a fact observed previously by R. Chambers), K_2SO_4 injections failed to liquefy the protoplasm though they did inhibit locomotion. These effects, just as the effects of $CaCl_2$ injections, were not influenced by acidification of the injected solutions (to a pH of 4.2–3.0). Since in the immersion experiments the K effect was the same irrespective of the anion used, these results support the previously suggested hypothesis that the plasma membrane of this amoeba is selectively permeable to cations and relatively impermeable to anions.

The efficiency of monochromatic ultraviolet radiation in the activation of Arbacia eggs. Alexander Hollaender.

During an investigation of the effects of ultra-violet radiation on the eggs of *Arbacia punctulata*, it was observed that when the eggs are exposed for as short a time as 1/10 of a second to the entire radiation of a water-cooled high pressure quartz capillary mercury vapor lamp, a large percentage of the eggs went through one or more cleavages without fertilization. Exclusion of the infra-red did not inhibit while exclusion of the radiation below 3,000A inhibited the effect.

The eggs were then irradiated with measured quantities of monochromatic radiation of nine different wave-lengths from 2,260 to 3,650A. Special care was taken to develop a method which would make certain that not only each egg within the dish but each part of each egg received an equivalent amount of energy. This was done by rotating a small dish in which the eggs were suspended in 3 mm. of sea water and blowing an air current against the water surface. The eggs were removed after irradiation to a larger volume of sea water and kept at 24° and 10° C.

Three types of controls were handled in each series of experiments: (1) unirradiated, unstirred eggs, (2) eggs stirred in the usual manner but protected against the radiation, (3) sea water irradiated with the wave-lengths most effective in producing activation to which unirradiated eggs were afterwards added. In none of these controls could any activation be recognized if the original eggs were in good condition.

The energy was measured with a standardized vacuum thermopile-galvanometer set up and the incident energy per egg calculated taking into account the total energy entering the dish, the time of exposure, the diameter of the egg and the fact that the eggs were exposed interruptedly ignoring for the present the energy reflected and scattered by the eggs.

Three to five hours after irradiation a high percentage of the eggs (up to 98 per cent) were found activated (one or more cleavages), if certain wave-lengths and energies per egg were used. The wave-length found most effective was 2,260A, and the efficiency decreased with the increase in wave-length, becoming negligible around 2,500A; 0.13 to 0.25 erg of incident energy per organism at 2,260A produce the highest percentage of activation. A plot of the percentage cleaving against the energy applied for each effective wave-length shows a typical S-shaped curve, a definite plateau, and finally a decreasing rate of cleaving with further increase of energy.

A plot of the efficiency of radiation against wave-lengths with its most pronounced maximum at 2,260A and its low efficiency at 2,600A suggests interesting comparisons with the ultra-violet inactivation spectra of urease and the virus of typical tobacco mosaic, and the absorption spectra of other protein-like substances.

Activation of centrifuged whole eggs of Arbacia and their fractions by monochromatic ultra-violet radiation. Ethel Browne Harvey and Alexander Hollaender.

Whole *Arbacia* eggs which have been stratified and elongated by centrifugal force (10,000 x g for 3 minutes) are activated by the same ultra-violet radiation as the whole uncentrifuged eggs; full arc 1/10–5 seconds, monochromatic 2,350–2,480A for 2–8 minutes. They behave in exactly the same way as when activated by other parthenogenetic agents such as hypertonic sea water but pass through only a few cleavages.

White half-eggs (nucleate) are activated by the same radiation but are much more sensitive to slight variations from an optimum treatment, and fewer cleave.

Red half-eggs (non-nucleate) are activated by the same radiation and also by a band of longer wave-length, 2,650–3,050A for 4–12 minutes, which does not affect the whole eggs and the white halves. Fertilization membranes are formed, some large asters occur, the egg often becomes aspherical and somewhat amoeboid. A notch frequently appears at the equator of the more spherical red halves, indicating the beginning of a cleavage plane, and this usually completes itself. Stages with 8–12 cells have been observed usually unequal in size. There occur later on some eggs filled with many small asters, a possible precursor to a blastula.

Yolk quarters (non-nucleate) are activated in just the same way as the red halves and by the same wave-lengths and dosage, and some 8–12-celled stages have been observed. The pigment quarters (non-nucleate) show some evidence also of being activated, since they form an ectoplasmic layer and become somewhat amoeboid.

Ultra-violet radiation, therefore, acts upon the whole eggs and their fractions obtained by centrifugal force exactly like other parthenogenetic agents such as hypertonic sea water. Since the radiation affects the non-nucleate fractions as well as the nucleate, the action must be on the cytoplasm, but since many abnormal and irregular mitoses are observed in stained sections of later cleavages, the action must be also on the nucleus.

The cytology of Arbacia punctulata activated by monochromatic ultra-violet radiation. B. R. Nebel, Ethel Browne Harvey and Alexander Hollaender.

Unfertilized eggs of *Arbacia* treated in the summer of 1936 with the full output of a high pressure mercury vapor lamp for a few seconds, showed activation in all the eggs and fairly normal first cleavages following the normal nuclear changes in 80 per cent of the eggs, but the cleavage was delayed by two or more

hours. A very characteristic feature of late prophase and metaphase stages in later cleavages as shown in prepared sections is the presence of small rings or spheres among the chromatic material. Occasionally the spindles of more than two plates appear united at various angles. Large cytasters are observed. Regular dicentric spindles, typically non-astral, occur but the most frequent type is a monaster surrounding the chromosomes, which as the division proceeds appears to form two polar half-asters. The eggs developed usually only to about an 4 to 8-cell stage.

Unfertilized eggs treated in the summer of 1937 with measured intensities of monochromatic ultraviolet of 2,350 and 2,260A for 4 to 6 minutes show up to 98 per cent activation indicated by a fertilization membrane and ectoplasmic layer and after 3-4 hours cleavage. Nuclear divisions are monastral and irregular, normal dicentric asters not being formed. The first division is frequently of the restitution type leading to diploidy without cell or nuclear division. The length of the spindle is diminished. During subsequent stages the products of successive chromatic multiplication may be separated. Thus irregular blastomeres may be formed containing no chromatin, approximately haploid, diploid or polyploid nuclei. The average activation does not produce more than 4 to 6 nuclei containing approximately variable multiples of the haploid chromosome number, distributed irregularly in 2 to 8 asymmetrical blastomeres.

Unfertilized eggs treated with the same and larger total energy given as ultraviolet $\lambda = 2{,}480$, 2,650 and 3,050A showed no marked activation.

Fertilized eggs treated with ultraviolet of the short wave-length (2,260 and 2,350A) soon after insemination showed slight inhibition. Fertilized eggs treated with ultraviolet of longer wave-length (2,650 and 3,050A) showed marked inhibition. In both treatments occasional nuclear irregularities were observed.

The relationship of sperm extracts to the fertilization reaction in Arbacia.

John A. Frank.

A specific egg-agglutinin, previously reported, is present in the filtrate from boiled *Arbacia* sperm suspensions. This substance is present in the fat-free residue on extracting sperm suspensions with alcohol and ether. It is not found in the lipid extract.

Eggs fertilized in sperm extracts show a marked drop in fertilizability when compared with eggs fertilized in sea water. Experiments were performed to determine whether this inhibition of fertilization is due to the action of sperm extracts on the egg alone, spermatozoön alone, or on both gametes.

Sperm suspensions exposed for varying lengths of time to sperm extracts exhibit a marked loss in fertilizing power. Sperm extracts thus block fertilization by a rapid direct action on sperm.

The fertilizability of jellyless eggs exposed to sperm extracts and subsequently fertilized with fresh sperm decreases markedly. Sperm extracts therefore exert an inhibitory effect on the cortex.

When sperm extract is added to egg water containing fertilizin, the mixture will not agglutinate sperm. Some substance in the sperm extract has inactivated fertilizin. Sperm extracts inactivate fertilizin in definite quantitative proportions. The capacity for fertilizin inactivation varies directly with the concentration of sperm extract. Thus sperm extracts contain a substance which resembles Lillie's anti-fertilizin.

On ageing, the fertilizing power of sperm suspensions is lost concurrently with the capacity of extracts of these suspensions to inactivate fertilizin and to agglutinate eggs.

Evidence at present indicates that sperm extracts contain substances related in some way to the fertilization reaction.

Stimulation and nuclear breakdown in the Nereis egg. L. V. Heilbrunn and Karl M. Wilbur.

Recent studies of stimulation in diverse types of protoplasmic systems have indicated that one of the primary effects is a breakdown of a calcium proteinate gel in the cell cortex and a release of free calcium into the cell interior. As yet these studies of stimulation have thrown no light as to the rôle the nucleus may play when a cell is stimulated to divide. In the egg of the worm *Nereis*, various types of stimulating agents cause a breakdown of the germinal vesicle. Thus, such an effect is produced by heat, ultraviolet radiation, and Roentgen rays, as well as by various chemicals. If our theory is correct, one of the initial steps in the series of processes that result eventually in nuclear breakdown is a calcium release from the cortex of the cell. Our experiments lend support to this theory. If *Nereis* eggs are exposed for 6 or more minutes to an isotonic citrate solution, subsequent treatment with ultraviolet radiation causes no nuclear breakdown; although on return to sea water the eggs again show a typical response following irradiation. Similarly previous treatment with citrate prevents the nuclear breakdown which otherwise occurs very beautifully when eggs are placed in isotonic solutions of sodium or potassium chloride. The sodium and potassium ions appear to be capable of provoking a release of calcium ions from the cell cortex, and these calcium ions induce changes which eventually lead to a breakdown of the nuclear membrane.

The movement of the egg nucleus in relation to the sperm aster in Lytechinus and Echinarachnius. Edward L. Chambers.

The observations indicate first that the egg nucleus is moved to the center of the sperm aster by centripetal currents of cytoplasm. This is in conformity with the early observations of Conklin. He held that the approach and union of the two nuclei were determined by protoplasmic currents in the oöplasm.

The granules in the cytoplasm move along with the egg nucleus.

The curvature of the path of the egg nucleus is caused by the continual change in direction of the cytoplasmic currents due to the progressive movement of the sperm aster towards the center.

The increasing acceleration of the egg nucleus indicates the existence of more and more intense centripetal currents of cytoplasm as the pronucleus migrates into the aster.

These observations indicate, second, that the aster is a jellied mass. This confirms R. Chambers' conclusion.

The diminishing acceleration of the egg nucleus as it moves down the ever-narrowing cytoplasmic path extending from the margin to the center of the aster demonstrates a resistance to movement due to the presence of a jellied material around the path through the aster.

The deformation (ellipsoidal) of the egg nucleus as it moves along this path demonstrates that the path is gradually tapering cone of fluid cytoplasm in the jellied mass of the aster.

The physical state of the wall of the furrow in a dividing cell. Robert Chambers.

Evidence is accumulating that the wall of the furrow is solid and constitutes the most solid part of the cortex of the cell. The advance of the furrow displaces the interior toward the poles which bulge because of the relative weakness of the polar cortex.

No symmetrical arrangement of the surface seems to be essential since strands of cortical protoplasm may be dragged out either at the equatorial or polar regions

without affecting the cleavage. The strands at the equatorial region are much stiffer than those at the poles. In tissue cultures fibroblasts often retain extended strands especially at the poles while the furrow forms at the equator.

Conditions within the cell interior need not affect the division once cleavage is under way. A dividing epithelial cell sometimes shows a rhythmic back-and-forth flow of the internal contents through the narrowing stalk connecting the two daughter cells. This ceases only when the constriction of the stalk has completed the division. In the sea-urchin egg undergoing division in calcium-free sea water, the contents of one blastomere, when torn, pour out while the furrow continues to deepen. The continued pinching-down of the furrow on the connecting stalk frequently rescues the other blastomere from disintegration.

The thickness of the cortex of the advancing furrow and the force with which it advances has been determined by injecting an oil drop into the equatorial region. The oil drop tends to come to lie in the central region at the equator so that the advancing furrow closes down on it and constricts it in two. This occurs when the surface of the floor of the furrow is some distance from the surface of the oil-drop.

The advance of the wall of the furrow must be considered as a growth phenomenon—material being added progressively to the gelated cortex of the furrow analogous to the apposition of material along the plane of division of a plant cell.

AUGUST 27

Chromosome studies in sundew (Drosera). A. Orville Dahl.

For purposes of comparison with chromosomes in certain members of the Saxifragaceae, cytological material of *Drosera filiformis* Raf., *D. longifolia* L., and *D. rotundifolia* L. has been locally collected. A preliminary examination of root-tip meristem in aceto-carmine demonstrates 20 chromosomes in *D. filiformis* which is consistent with Levine's (*Mem. N. Y. Bot. Gard.*, 6: 125–147, 1916) report of 10 chromosomes in pollen mother-cell material from Lakehurst, N. J. Rosenberg (*Ber. der Deutschen Bot. Ges.*, 21: 110–119, 1903) found 20 chromosomes in cells of root-tips, stems, leaves, and flowers of *D. rotundifolia* collected in Germany, Norway, and Sweden. A comparative study of the early somatic metaphase in *D. filiformis* from Mashpee, Mass. and *D. rotundifolia* from North Falmouth, Mass. shows that the chromosomes of the former are approximately 1.49 times longer and 1.38 times wider than those of the latter. The metaphase chromosomes are of comparatively small size, those of *D. rotundifolia* being about 1.90 μ in length while those of *D. filiformis* are 2.82 μ in length. A visibly four-parted structure, with the distance between the half-chromatids 0.3 μ to 0.4 μ, could be detected at mid-prophase, late prophase (at which time the nucleus has a diakinetic appearance), early metaphase, and late telophase.

Mitosis in the giant amoeba, Chaos chaos Linnaeus. M. Catherine Hinchey.

Chaos chaos is from 50 to 500 times as large as *C. diffluens* and has from 50 to over 1,000 discoid nuclei. The chromatin granules are arranged in a layer underneath the nuclear membrane in the living amoeba.

When mitosis begins, the nuclei become spherical and the chromatin granules congregate in a thick equatorial plate. Then most of the granules seem to disappear, leaving a spherical nucleus, with chromatin granules distributed in a thin plate. What appear to be spindle fibers become visible at this stage. These fibers can be seen in the living amoeba under the micro-compressor.

Following this stage, the nucleus shortens along the polar axis and becomes wider at the equator, so that a ladder of fibers is seen, in optical section, with the chromatin distributed along the mid-points of the ladder-rungs.

The chromatin then separates into two plates which move along the spindle fibers toward the poles, until the plates are three times their diameter apart. At this stage fibers can be plainly seen in both stained and living material, extending between the plates and from the plates to the poles.

Protoplasmic streaming next moves the plates apart. The fibers between the plates become twisted and disappear, but the fibers going to the poles persist for some time longer. The chromatin plate becomes thinner, and so homogeneous that it is extremely difficult to follow in living material, but in fixed material, a concentration of chromatin occurs along the inner edge of the plate. The daughter nuclei next become wider and more granular. This process continues by gradual steps until the interphase stage is reached.

Cytoplasmic division—usually into 3 daughters—occurs during the reorganization of the daughter nuclei into interphase nuclei.

The striking features of the nuclear division are: 1. All the nuclei divide at the same time. 2. Practically every stage of mitosis can be seen in the living amoeba under the micro-compressor. 3. Although the total number of nuclei doubles during mitosis, cytoplasmic division usually results in three daughters.

Some effects of oxygen on polarity in Tubularia crocea. James A. Miller.

A chamber was constructed by means of which the two ends of *Tubularia* stems could be exposed to different agents or to different concentrations of the same agent. This consisted of a double chamber with a partition which separated the solutions but which had perforations through which the stems could be passed. Using this apparatus preliminary studies were made upon the effects of high and low oxygen tensions on polarity. By placing the stems in alternating orientations each experiment served as its own control. Oxygen determinations by the Winkler method were made in all but preliminary experiments.

When oxygen was bubbled on one side of the partition and boiled sea water was placed on the other, hydranths developed only on the side with high oxygen. One half of these were distal and the other were proximal hydranths. Similar results were obtained when oxygen was bubbled on one side of the partition and standing sea water (with 4.1 to 5.0 cc. O_2 per liter) was on the other. That these results were caused primarily by the oxygen differential and not by a possible accumulation of carbon-dioxide was demonstrated when nitrogen was bubbled on one side of the partition and oxygen on the other. Here again there was a reversal of polarity in all stems with their proximal ends exposed to the oxygen.

The importance of circulation of the medium to sessile forms such as *Tubularia* was illustrated by experiments in which 95 per cent of the stems developed proximal hydranths in running aerated water when the distal ends were exposed to standing water, while only 6.3 per cent developed proximal hydranths when the conditions were reversed. Oxygen determinations in two of these experiments showed a difference of only 0.1 cc. per liter in each case.

Some effects of strychnine on reconstitution of hydranth primordia in Tubularia crocea. Faith Stone Miller and James A. Miller.

Miller (1937) found that pieces of planarians regenerating in strychnine showed no evidence of stimulation. Since in *Tubularia* the size and time of development of hydranth primordia can be measured, this form was used to continue the study of the effects of strychnine.

The behavior of unoperated individuals placed in solutions of strychnine sulphate in sea water was undistinguishable from that of controls anesthetized by magnesium sulphate.

In reconstitution experiments stems selected for uniform appearance and diameter were used. Ten-millimeter pieces were cut with the distal end five millimeters from the base of the hydranth. Continuous exposure resulted in a decrease in size of hydranth primordia and increase in time of development. Concentrations used ranged from M/20,000 which showed very little effect to M/5,000 in which few stems survived. Temporary exposures to M/1,000 for periods of ½ to 8 hours showed similar but less consistent results. The frequency of the occurrence of bipolar forms was decreased in strychnine and none developed in the higher concentrations.

The results obtained with strychnine on *Tubularia* are similar to those with inhibitory agents and indicate that it produces a definite depression. By bubbling oxygen through the solutions it was possible to antagonize the strychnine effect.

The life cycle of Moniezia expansa. Horace W. Stunkard.

Anoplocephaline cestodes are common parasites of herbivorous animals and one species is recorded from man. They are worldwide in distribution, and the group has been intensively studied for more than fifty years. The final hosts harbor sexually mature worms in their intestines, eggs of the parasites are voided with the feces of the hosts, but what occurs in the interval before the cestode reappears in the intestine of the primary host has remained quite unknown.

Stunkard (1934) published results of experiments which demonstrated that the final hosts could not be infected with eggs of the parasite and that an intermediate host is necessary for the completion of the life cycle.

Experiments have been continued, using species of *Moniezia* from sheep and *Cittotaenia* from rabbits. Various minute, terrestrial invertebrates, chiefly insects, have been used in attempts to discover the intermediate hosts of these cestodes. In the spring of 1936, tyroglyphid mites were fed eggs of *Moniezia* and onchospheres were recovered from the body cavity three days later. The structure, habits, and life history of these mites indicate that they would not be suitable intermediate hosts of *Moniezia*. The oribatid mites, however, appeared to be admirably suited, and representatives of this family were employed. *Galumna sp.* are abundant in regions where *Moniezia* occurs and specimens of this mite were collected from areas in which there were no sheep. These mites were fed eggs of both *Moniezia* and *Cittotaenia* and onchospheres of both species were recovered from the body cavity. During the past year thousands of *Galumna sp.* have been fed eggs of *Moniezia expansa* and a series of developmental stages, from the onchosphere to the mature cysticercoid, have been recovered from them.

A new method for studying the pH of the intercellular substance in the living mammal. Richard G. Abell and Eliot R. Clark.

This method involves the installation of phenol red within a transparent moat chamber in the ear of a rabbit. Such a chamber contains a thin space, called the 'bay,' into which living tissue, continuous with the subcutaneous tissue of the ear, grows through two small entrance holes at the proximal end. The bay has a glass bottom and a mica top, and is only 50μ to 100μ deep. Consequently the arterioles, capillaries, and venules, and other constituents of the tissue within it can be seen clearly with the microscope. At its distal end the bay opens directly into a reservoir, called the 'moat.'

Following the introduction into the moat of a 0.4 per cent solution of phenol red, made isotonic with rabbit's blood by the addition of NaCl, the indicator diffuses into the bay, and there colors diffusely the intercellular substance of the

tissue for a distance of approximately 1 mm. proximal to the most advanced capillaries. It is not concentrated by the cells, and it is not toxic.

The color of the indicator in the intercellular substance of tissue with an active circulation is pink, the shade of pink matching that of a phosphate buffer at pH 7.2, to which phenol red has been added, seen with a microscope in the counting chamber of a hemocytometer under the same conditions of illumination as used for the tissue.

When the circulation is cut off, by compressing the main artery of the ear, the color of the intercellular substance changes to the orange-yellow of a buffer at pH 6.8 within 10 to 15 minutes, indicating accumulation of acid metabolites. Within 1 to 2 minutes after the artery is released and the circulation once more becomes active, the color of the intercellular substance changes from orange-yellow back to pink.

By means of the present method, the pH of the intercellular substance can be studied under a variety of experimental conditions.

The behavior of living mammalian arterioles, capillaries, and venules when exposed to CO_2. Richard G. Abell and Eliot R. Clark.

The experiments to be described were performed in a transparent moat chamber in the ear of a rabbit. The behavior of the vessels was studied with the microscope, and changes in the pH of the intercellular substance detected by means of the indicator method presented above.

When CO_2 is passed through the moat, the color of the phenol red in the intercellular substance of the tissue in the bay turns from pink (pH 7.2) to yellow (pH 6.8–6.6). No increase in stickiness of the endothelium occurs when the amount of CO_2 employed is small. If the endothelium is sticky toward leukocytes before CO_2 perfusion is started, it reverts to a state in which the leukocytes roll freely along the vessel walls.

No increase in the diameter of the arterioles, capillaries, or venules occurs as long as the circulation continues to pass through them. The arterioles in these experiments were not supplied by nerves.

The color of the indicator in the intercellular substance of the proximal tissue turns from pink to yellow more rapidly when the circulation is sluggish than when it is rapid. Such color change is followed shortly by thickening and vacuolization of the endothelium of the arterioles.

If CO_2 perfusion is stopped at this stage, the vacuoles disappear, and the endothelium resumes its normal thickness. If perfusion is continued, a marked increase in permeability of the arterioles, and also of the capillaries and venules, occurs. The plasma passes through the walls of the vessels, leaving within their lumina only the formed elements of the blood. During this process extensive crenation of erythrocytes occurs. In vessels containing concentrated cells, the flow of blood is blocked.

No increase in diameter of the arterioles, capillaries, or venules occurs at the time of onset of plasma hemorrhage, but may take place shortly thereafter, indicating a softening of the endothelium. These changes are reversible if CO_2 perfusion is stopped when plasma hemorrhage first occurs.

The control of peripheral circulation in the mammal. Eliot R. Clark and Eleanor Linton Clark.

It has been possible, in transparent chambers introduced in the rabbit's ear, to watch the mode of formation and behavior of extra-endothelial cells, with the following results:

Fibroblast-like cells from outside the endothelium become flattened out on the wall of the capillary at a very early stage in capillary formation—often during

sprout formation. If the vessel remains a capillary, there may be no increase in their number and they may remain permanently as sparsely distributed inert, oval cells. If the capillary becomes a portion of an artery the number of outside cells increases rapidly—in part at least by mitotic division—the axis of the cells changes quickly from a longitudinal to a transverse position and muscle cells develop which show typical contractility in case they receive a nerve supply.

If the capillary becomes a part of a venule or small vein, the number of adventitial cells increases slightly, their long axis remains longitudinal and they do not develop contractility.

Persistent observations of the living vessels made under a great variety of conditions has corroborated earlier findings that, in the mammal, neither the endothelium nor the adventitial cells, as found on the capillary or venule, manifests active contractility. The control of the peripheral flow resides in the muscle cells of arteries, arterioles and certain of the veins—providing the muscle cells are under nerve control.

While there may be changes in the caliber of capillaries and bulgings into the lumen of endothelial nuclear thickenings or of adventitial cells, all such changes are apparently passive, secondary to a variety of factors, chief of which are changes in internal pressure and rate of flow, produced by contraction or dilatation of supplying arteries or arterioles, and changes in outside pressure occasioned either by variations in the amount of fluid accumulation in the intervascular spaces or by the elasticity of the enclosing wall.

The structure of the liver lobule. Louis Loeffler.

The liver of the pig is the only one which shows clearly defined lobules. By that is meant that the lobules have a connective tissue membrane separating one lobule from another. The capillaries, also, of an individual lobule are separated from the capillaries of the adjacent lobules. All the other livers of mammals, reptiles and fishes, so far as has been investigated, show no separating membranes and also show anastomosing capillaries throughout the whole organ. Nevertheless, one is justified to speak of liver lobules, because the vessels, situated in regular distance from one another, form figures similar to the pig liver lobules. It is shown, however, that there are no so-called sub-lobular veins, because such veins, next in size to the central veins within each lobule, function as central veins quite the same. Hepatic veins come to lie outside the lobules, not before. The hepatic veins reach a diameter half or much more than that of a liver lobule. A collecting lobe of about 6 or 8 lobules around a sub-lobular vein as usually found in the diagrams of anatomical textbooks does not exist or is quite arbitrary. The pig's liver should be explained on the basis of a physiological liver cirrhosis.

A preliminary note on the innervation of the swim-bladder of the sea-robin. John B. Gaylor and Ernst Scharrer.

This communication deals with the peripheral innervation of the sea-robin as investigated by Dr. Gaylor at Woods Hole; a combined paper will be published later when Dr. Scharrer has worked out the central connections.

The swim-bladder of the sea-robin consists of a two-lobed sac in the abdominal cavity. It possesses intrinsic skeletal muscle which subserves the function of noise production and, in the interior of the cavity, a gas gland which is in the form of a "rete mirabile" covered with secretory epithelium. A branch of the vagus on either side affords a motor supply to the striated musculature. Free endings, knob endings and ring terminations have been observed. There is no apparent difference in the mode of termination in the swim-bladder muscle from

that of the usual somatic musculature. Ganglia in the striated muscle are sparse; the disparity between the wealth of fiber and the number of ganglia argues a double innervation to the skeletal muscle—one direct from the vagal nucleus and one relayed through peripheral ganglion cells. The presence of muscle-spindle organs in the region where the muscle takes origin from the fibrous sac is suspected but not yet definitely established.

The "rete mirabile" contains fine non-medullated fibers which enter along with the vessels and which appear to be sympathetic. Between the vessels there is a large number of multipolar ganglion cells which are presumably parasympathetic relays.

The origin and development of the thyroid in Eleutherodactylus, an anuran with no tadpole stage. W. Gardner Lynn.

The Jamaican tree-toad *Eleutherodactylus nubicola* lays its eggs on land and the young hatch after about twenty-four days with a definitive body form. During the embryonic development some of the larval characters which are usually found in frogs appear very transiently but others, such as the formation of external and internal gills, are entirely lacking. A study has been made of the thyroid in embryos preserved at twenty-four-hour intervals throughout the period of development. The thyroid takes its origin from the pharynx at the sixth or seventh day and the cells exhibit signs of secretory activity almost as soon as the thyroid anlage is definitely recognizable. Throughout the succeeeding stages there is a steady increase in the amount of stored colloid. Intracellular vacuoles are abundant in the follicle cells at all stages. Vacuolation of the colloid mass is most striking during a period of about four days extending from the tenth to sixth day before hatching. There is no evidence of a sudden release of any large amount of colloid into the blood stream at any time during the embryonic history. The indication is, rather, that a regular release occurs even from the early stages. This would agree with the regular course of bodily differentiation and the absence of any striking metamorphic pattern. However, certain of the unusual features of development in this frog, such as the absence of external gills, cannot be attributed to precocious thyroid functioning. Thus it appears that while the evolutionary changes which have brought about the atypical life history of the species are some of them changes in the development and functioning of the gland complex, still others are changes which cannot be attributed to hormonal influence but are direct alterations in the developmental potentialities of the tissues themselves.

Some effects of mammalian follicle-stimulating and luteinizing hormones in adult female urodeles.[1] Virginia Mayo.

During October adult salamanders (*Triturus viridescens*) were subjected to preliminary tests on their ovulatory response to a single intraperitoneal injection daily of the following mammalian pituitary extracts made according to H. L. Fevold's method: (*a*) physiologically pure follicle-stimulating hormone (F.S.H.); (*b*) physiologically pure luteinizing hormone (L.H.); (*c*) F.S.H. with a trace of L.H.; (*d*) an unfractionated extract containing both F.S.H. and L.H. Each injection contained 1/20 gram-equivalent acetone-dried sheep pituitary powder. The results indicated that fractions containing L.H. induced egg-laying whereas F.S.H. alone was almost entirely ineffective. Injections given both normal and hypophysectomized newts in November–December, May–June, and June–July–August corroborated the October findings.

[1] This work was begun at the Biological Laboratories, Harvard University, and continued at the Marine Biological Laboratory.

Counts of eggs released by groups of 10 animals treated during the breeding season for 40 days gave the following: hypophysectomized L.H.-injected animals ovulated 801 eggs; normal L.H.-injected, 582; hypophysectomized F.S.H.-injected, 18; and normal F.S.H.-injected, 25.

At the onset of the June–July–August series the newts' ovaries were almost completely emptied of large eggs. By early August the ovaries of F.S.H. and F.S.H. + L.H.-treated animals were filled with yolked eggs, while those of L.H.-treated individuals were only slightly stimulated. Average ovarian weights of 7–14 animals were: untreated controls, 28 mg.; hypophysectomized + L.H., 69 mg.; normals + L.H., 88 mg.; hypophysectomized + F.S.H., 119 mg.; normals + F.S.H., 134 mg.; hypophysectomized + F.S.H. and L.H., 150 mg.; and normals + F.S.H. and L.H., 173 mg.

On the basis of these results it seems that F.S.H. brings about a striking enlargement of the ovary while the L.H. is primarily responsible for egg release

The relation of melanophore responses to vascular disturbances. G. H. Parker.

It is difficult to cut nerves in experiments on melanophore control without at the same time cutting blood-vessels or at least introducing vasomotor disturbances. Does the stimulation of melanophore nerves thus brought about excite vasomotor changes which in turn excite responses in melanophores or do the nerves act directly on the melanophores? In the killifish *Fundulus* and in the catfish *Ameiurus* the melanophores have a double innervation, concentrating nerve-fibers inducing a blanching of the fish through a concentration of pigment in its melanophores and dispersing fibers darkening the fish through a dispersion of this pigment. In the dogfish *Mustelus* there are only concentrating fibers, the dispersion of the pigment being accomplished through a pituitary neurohumor in the blood.

In *Fundulus* and *Ameiurus* when melanophore nerves are cut the dispersing nerve-fibers are stimulated and dark areas or bands result. In these two fishes and in *Mustelus* when the melanophore nerves are stimulated electrically the concentrating fibers are excited and the fishes blanch locally.

One way of finding an answer to the question under consideration is to ascertain whether these responses will occur or not in the absence of an active circulation of blood. To this end the ventral aorta of a given fish was ligated just anterior to the heart and the circulation of blood thus brought to a complete standstill. The melanophore nerves of such a fish were then subjected to electric stimulation or were cut. In all such instances there was either local blanching or darkening according to the stimulus. These responses, though less marked than in normal fishes, showed unquestionably that the complete loss of circulation was not accompanied by a loss of the power of color response and that therefore vasomotor or other vascular changes could not form any essential part in the chain of events between nerve and melanophore.

Some effects of chloroform on the respiratory systems of yeast. E. P. Hiatt and J. K. W. Ferguson.

The rate of reduction of methylene blue in suspensions of yeast is increased by small amounts of chloroform (0.05 per cent by weight). With larger amounts of chloroform, acceleration up to 40 times was obtained. No stage of inhibition was reached with fresh yeast. Dried yeast and yeast extracts, which have a much faster rate of reduction than equivalent amounts of fresh yeast, were retarded by chloroform. The same effects can be demonstrated with other oxidation-reduction indicators, e.g., pyocyanin, thionin, and anthraquinone.

When chloroform was exerting its maximal acceleration, the cytochrome bands showed a characteristic change. Only the C band was visible and it could not be made to disappear on shaking with air. Subsequently, different amounts of chloroform were found to affect the cytochrome spectrum differently. With concentrations up to 0.1 per cent by weight, the time for the appearance of all three bands (rate of reduction), was shortened. At about 0.3 per cent the A band disappeared, the B and C bands appearing slowly and remaining fixed. At about 0.5 per cent the B band disappeared.

Similar but less marked effects were obtained with ether and 95 per cent ethyl alcohol. It is interesting to note that carbon tetrachloride has little effect.

The oxygen consumption of the chloroform-treated yeast, as determined by the Warburg technique, was also greatly increased;—up to 20 times. Maximal acceleration was obtained in a medium of phosphate buffer at pH 6.6. Less effect was noted at pH 7.0 and little or none at 8.5. A similar acceleration was observed with fertilized and unfertilized *Arbacia* eggs.

In view of the prevalent idea that narcotics act by depressing cellular respiration, it seems significant that these accelerating effects on respiration were obtained with concentrations of the same order of magnitude as are effective in producing general anesthesia.

The oxygen consumption of activated and fertilized eggs of Chaetopterus. Jean Brachet.

Unfertilized *Chaetopterus* eggs undergo activation when they are treated with 5 per cent isotonic KCl in sea water; maturation is followed by a series of monasterian cycles leading to the formation of unicellular larvae resembling gastrulae and trochophores (F. R. Lillie's differentiation without cleavage). The oxygen consumption of these activated eggs has been compared with the respiration of unfertilized and of fertilized eggs during 7 hours (Warburg's method). Activation is followed by a considerable drop in the oxygen consumption (49 per cent); and fertilization has exactly the same effect, as was shown first by Whitaker. The O_2 uptake increases then, but at a much slower rate in the activated eggs than in the fertilized ones: while these resume their initial respiratory rate after 3½ hours, the activated eggs need 6 hours to reach that level. The respiration of the unfertilized eggs remains constant for 7 hours. Control experiments showed that isotonic KCl in the concentration of 5 per cent used has no significant effect on the metabolism of *Chaetopterus* eggs: the slope of the curve is not changed if the KCl treated eggs have been repeatedly washed or when KCl is added to the fertilized eggs. The reduced metabolic activity of the activated eggs must thus be linked to either their slower development or to the fact that they remain unicellular.

Influence of respiratory inhibitors on stimulation of metabolism by nitro and halo phenols. M. E. Krahl, Anna K. Keltch and G. H. A. Clowes.

At the 1934 meeting, experiments reported from this laboratory showed that the respiratory stimulation produced by 4,6-dinitro-*o*-cresol in fertilized eggs of *Arbacia punctulata* was progressively inhibited and could be completely abolished by increasing concentrations of potassium cyanide and that the division-blocking effects of the two reagents were additive. During the past three seasons these experiments with cyanide and 4,6-dinitro-*o*-cresol have been extended and similar experiments made with other respiratory inhibitors.

The following concentrations of inhibitors have been found, with eggs in sea water at pH 8, give a suppression of normal respiration which is just measurable (i.e., 5 to 20 per cent): CO, 94CO : $6O_2$; 'Amytal' (Iso-amyl Ethyl Bar-

bituric Acid, Lilly), 2×10^{-4} M; malonic acid, 10^{-3} M; and, at pH 6, iodoacetic acid, 10^{-4} M. At these same respective concentrations of the inhibitors there is a progressive decrease in the extent to which the various inhibitors suppress the respiration stimulus by 4,6-dinitro-*o*-cresol, the first two members of the series giving almost complete, and the last two members almost negligible suppression of the stimulated respiration. With the exception of phenyl urethane and malonic acid, with which such experiments were not made, all inhibitors used gave complete suppression of stimulation by 4,6-dinitro-*o*-cresol when tried in sufficiently high concentrations. With partial inhibition by CO or KCN, the optimum stimulation of the residual respiration was produced by concentrations of 4,6-dinitro-*o*-cresol larger than those required in the absence of CO or KCN. With partial inhibition by the other inhibitors, optimum stimulation of the residual respiration was produced by concentrations of 4,6-dinitro-*o*-cresol equal to or less than those required in the absence of inhibitor.

In this series of experiments, the concentration of 4,6-dinitro-*o*-cresol required, in the absence of inhibitors, to give 90 to 100 per cent block to division was 8×10^{-4} M. The concentrations of 4,6-dinitro-*o*-cresol required in the presence of the concentrations of inhibitors mentioned above, which alone gave little or no block to division, were 4×10^{-4} M in CO; 8×10^{-4} M in low oxygen tension; less than 10^{-4} M in KCN; 8×10^{-5} M in malonic acid.

Substituted phenols as inhibitants of the fertilization of Arbacia and of ciliary movement of Arenicola larvae. G. H. A. Clowes, M. E. Krahl and Anna K. Keltch.

It has already been demonstrated for a considerable series of nitro and halo phenols that the point of concentration required for maximum stimulation of respiration corresponds approximately with the point at which cell division is blocked in fertilized sea urchin eggs. In an attempt to throw further light on the nature of the mechanism involved, the concentrations were determined at which certain representative nitro and halo phenols blocked the fertilization of *Arbacia* eggs by sperm and anesthetized *Arenicola* larvae. The block to fertilization and anesthesia of larvae occurred at about the same concentration for each individual compound, but these concentrations differed in certain cases very greatly from the concentrations at which the respiration peak and cell division block occurred.

In the case of 2,4-dinitrophenol and 4,6-dinitro-*o*-cresol, having pK values of 4.1 and 4.4 respectively, the ratios of concentration required for anesthesia to that required for internal cell division block were found to be 137 : 1 and 228 : 1. That for 2,4,5-trichlorophenol, having a pK of 6.9, was found to be 19 : 1, whilst

Concentrations (moles per liter $\times 10^5$) of substituted phenols required to inhibit various physiological functions. pH 8.0.

Compound	I pK	II Ciliary Movement Arenicola	III Fertilization Treated Eggs Treated Sperm	IV Cell Division Arbacia	Ratio III : IV
2,4-Dinitrophenol	4.1	205	410	3.0	137 : 1
4,6-Dinitro-*o*-cresol	4.4	205	205	0.9	228 : 1
2,4,5-Trichlorophenol	6.9	13	26	1.4	19 : 1
o-Nitrophenol	7.2	No effect	No effect	No effect	—
2,4-Dichlorophenol	7.7	51	26	26.0	1 : 1
m-Nitrophenol	8.3	205	205	51.0	4 : 1

those for 2,4-dichlorophenol, having a pK of 7.7 and *m*-nitrophenol, having a pK of 8.3, were found to be 1:1 and 4:1. It is particularly interesting to note that orthonitrophenol, which although proved to enter the cell, had no effect on cell division or oxidation, had also no effect on the fertilization process or on ciliary movement of *Arenicola*. It appears advisable to refrain from speculation regarding these results until further data are available.

Stimulation of the rate of cell division of Arbacia eggs by carcinogenic hydrocarbons. Anna K. Keltch, M. E. Krahl and G. H. A. Clowes.

As a part of an investigation into the mechanism by which certain polycyclic hydrocarbons produce cancer, a study has been made, during the seasons of 1935 and 1936, of the effects produced by three carcinogenic and two closely related non-carcinogenic hydrocarbons on cell division of fertilized eggs of *Arbacia punctulata*, using each hydrocarbon in the form of its water-soluble choleic acid, these being addition compounds of the hydrocarbon with desoxycholic acid.

Unfertilized eggs were exposed to varying concentrations of each choleic acid in sea water solution for varying periods of time. They were then fertilized, and left in the same respective solutions and at the same respective temperatures during the periods of pretreatment, fertilization and division, with the single exception that at 5° C., the eggs were raised to 15° for approximately five minutes in order to allow fertilization to take place and then returned immediately to 5° C., the controls in every case being subjected to treatment identical with that given the experimental material.

Typical representative results obtained with a five-hour pretreatment are presented in the accompanying table. In these data it is desired to emphasize—(*a*) that the choleic acids of the three hydrocarbons which produce cancer in mice also produce a shortening of division time and that the choleic acids of the two closely related hydrocarbons which produce no cancer in mice do not produce a shortening of division time; (*b*) that, with optimum concentration of 6-methyl cholanthrene choleic acid, there is a progressively smaller relative decrease in the division time as the temperature is raised from 5° to 15° C.

Choleic Acid	Temperature	Minutes to 50 Per Cent First Cleavage		Carcinogenic Activity in Mice
		Control	Minimum with Hydrocarbon	
	°C.			
6-methyl cholanthrene	5	1717	1482	Positive
6-methyl cholanthrene	10	235	221	
6-methyl cholanthrene	15	109	100	
10-methyl-1,2-benzanthracene	15	122	116	Positive
1,2,5,6-dibenzanthracene	15	126	123	Positive
Phenanthrene	15	118	118	Negative
Fluoranthene	15	115	115	Negative

The molecular species concerned in the action of substituted phenols on marine eggs. Albert Tyler and N. H. Horowitz.

In a recently published article (Tyler and Horowitz, 1937) the view was expressed that the substituted phenols penetrate as the undissociated molecule,

but exert their respiratory stimulating and reversible block to cleavage effects as the anion. The evidence for this rests on the fact that with any one of these compounds the concentration required for maximum effect varies with the pH of the solution, but the calculated concentration of the undissociated form present is the same at all pH's. This might mean that the undissociated form is the active species. However, when the various compounds are compared, the concentration of undissociated form at maximum effect shows enormous differences. On the other hand, when the comparison is made on the basis of calculated concentrations of the anion present inside the cell the various substances give values of the same order of magnitude. The compounds investigated include the three mononitrophenols, 2,4- and 2,6-dinitrophenol, 2,4,6-trinitrophenol, the three monochlorophenols, 2,4-, 2,5-, 2,6- and 3,5-dichlorophenol, 2,4,6-trichlorophenol and 2,6-dichloro-4-nitrophenol. Four of these; namely, *o*-nitrophenol, trinitrophenol, *o*-chlorophenol, and 2,6-dichlorophenol, show large deviations, but these substances are actually inactive or only slightly active in stimulating respiration.

Extension of the length of fertilizable life to more than twice the control is also obtained with dinitrophenol, confirming the findings reported by Clowes and Krahl. This prolongation occurs at high concentrations giving no respiratory stimulation. Determinations of the temperature coefficient of the respiration of unfertilized eggs shows it to be the same in dinitrophenol as in sea water.

Ovoverdin, a pigment chemically related to visual purple. Kurt G. Stern and Kurt Salomon.

The eggs of the lobster (*Homarus americanus*) owe their green color to a pigment belonging to the class of carotenoid-proteins. According to G. Wald[1] visual purple is another member of this widely distributed group of chromoproteids.

The carotenoid contained in the lobster egg pigment is astacin which is esterified with an as yet unidentified organic acid.[2, 3] This "ovoester" is in turn linked up with a protein of albuminoid character. The name ovoverdin is proposed for the native pigment complex.

Ovoverdin may be obtained in solution by grinding the eggs with sand and extracting them with distilled water. Treatment with an equal volume of saturated ammonium sulfate removes oil globules containing carotine and small amounts of globulins. The solutions may be further purified by repeated precipitation of ovoverdin in saturated ammonium sulfate solution or by dialysis at low temperature.

Ovoverdin has two absorption bands in the visible, at 6,400 and 4,700 A.; in addition it shows the typical protein absorption in the ultraviolet. The molecular weight, according to the rate of sedimentation in the ultracentrifuge as measured by Dr. R. W. G. Wyckoff, is of the order of 300,000. The isoelectric point is at pH 6.7.

Organic solvents and weak acids liberate the orange red carotenoid by virtue of denaturation of the protein carrier. When solutions of ovoverdin or the whole lobster eggs are rapidly brought to 65 to 70° the color turns from grass green to bright orange red. The red form shows increased light absorption at 4,700 A and greatly diminished absorption at 6,400 A as compared with the green form. When the heated material is rapidly cooled, the green color returns. This reversible thermal dissociation is different from an irreversible dissociation which takes place upon longer exposure to these temperatures or upon raising the temperature to the vicinity of the boiling point. At the latter point coagulation

[1] Wald, G., 1935–36. *Jour. Gen. Physiol.*, **19**: 351.
[2] Kuhn, R., and E. Lederer, 1933. *Ber. Deutsch. Chem. Ges.*, **66**: 488.
[3] Karrer, P., L. Loewe, and H. Huebner, 1935. *Helv. Chim. Acta*, **18**: 96.

of the protein occurs. The sequence of events and a photoelectric study of the color-temperature-time curves indicates a lower energy requirement of the first, reversible stage of dissociation as compared with the later, irreversible stages.

It has been suggested[3, 4] that the bleaching of visual purple by light is a disruption of the purple carotenoid-protein complex; the orange carotenoid, retinene, is liberated and the protein is denatured. This assumption, however, is open to the objection that the energy content of the effective wave-lengths of light is smaller than the energy required for inactivation of the visual purple complex. The present observations would suggest that the reversible bleaching of the retinal pigment does not involve a denaturation of the protein component but is rather of the type of the thermal phenomenon here observed. This hypothesis appears to receive support from the fact that the energy levels at which the latter occurs are lower than those at which protein denaturation takes place and secondly that the process observed here is rapidly reversible whereas protein renaturation in general is a time-consuming process and therefore not well suited for the regeneration requirements of visual purple during vision.

The increase of CO_2 and decalcification in certain pelecypods. Louis-Paul Dugal and Laurence Irving.

In three forms of pelecypods, *Venus mercenaria, Ostrea sp.* and *Elleptio complanatus,* the mantle cavity fluid gains CO_2 when they are kept out of water. The change is from 5 or 8 (in fresh ones) to 90 ml. per 100 ml. of fluid (for individuals kept out of water about 5 or 6 days). When the mollusks begin to die, the total CO_2 decreases. Return to water before death restores the CO_2 to normal. The accumulation of CO_2 probably results from a disturbance of respiration.

In *Venus,* which was most carefully studied, the total CO_2 of the M.C.F. increases rapidly. The pH decreases only from 7.4 to 7.2 and the P_{CO_2} increases only from 3 to a maximum of 25 mm. Hg, so that it is evident that the buffering capacity increases.

The shell is eroded during these changes and a few analyses showed that the mantle cavity fluid gained Ca, so that it is easy to guess that the buffering is provided by the solution of $CaCO_3$ from the shell.

The shell erosion is localized in the central inner part; the mantle tissue and no others, gain CO_2. This indicates that the buffering is effected by the activity of a special tissue.

The effect of pH and ionic strength on the activity of carbonic anhydrase. J. K. W. Ferguson and E. C. Black.

The manometric method of Meldrum and Roughton for determining the activity of carbonic anhydrase by following the rate of evolution of CO_2 from a mixture of phosphate and bicarbonate solutions, has been favored, because of its simplicity, for use in physiological and pathological studies. The reaction used in this method takes place in a medium of changing ionic strength and pH. As yet no adequate analysis of the effect of these variables on the activity of the enzyme has appeared.

In this preliminary study the CO_2 output method has been used and consequently the variation of pH was limited to the range from pH 5.7 to pH 8.0. The pH and ionic strength taken as corresponding to a certain activity was the mean pH and ionic strength of the range traversed by the stage of the reaction used in calculating the activity (usually from the beginning to two-thirds completion).

[4] Mirsky, A. E., 1936. *Proc. Nat. Acad. Sci.* (Washington), **22**: 147.

Between pH 6.0 and 8.0 an eight-fold increase in enzymic activity was found, the activity increasing with pH. Between ionic strengths of 0.5 and 0.1 at constant pH a 2.5-fold increase in enzymic activity was found, the activity increasing as the ionic strength decreased.

The distribution of carbon dioxide in dogfish blood. J. K. W. Ferguson, S. M. Horvath, and J. R. Pappenheimer.

After unsuccessful attempts to apply to fish bloods the chemical and kinetic methods of estimating carbhemoglobin, evidence concerning the state of CO_2 in erythrocytes of fish was sought by studying the distribution of CO_2 between red cells and plasma. In this study smooth dogfish (*Mustelus canis*) and a few specimens of spiny dogfish (*Squalus acanthius*) were used. Defibrinated or heparinized blood was used exclusively after it was found that both oxalate and fluoride grossly affected the distribution of electrolytes between cells and plasma.

The bloods were found to fall into two groups as regards total CO_2 capacity. During June and early July the CO_2 capacities at a CO_2 pressure of 40 mm. Hg. were on the average twice as great as in the bloods obtained during late July and August. In the early part of the season the curves were steeper, indicating greater buffer power. This difference could not be attributed to differences in hemoglobin content. The degree of oxygenation has no effect on the CO_2 capacity of dogfish blood.

Usually the concentration of CO_2 in the red cells (per unit H_2O) is greater than in the corresponding plasma. This is different from the situation in mammalian blood and inconsistent with the view that the bulk of the CO_2 in the cells is in the form of active bicarbonate ions. When chloride distributions were studied the contrast was striking. The ratio of intracellular to extracellular concentration of chloride, (r_{Cl}), is always much smaller than the similar ratio for CO_2, (r_{CO_2}). In mammalian blood $r_{CO_2} = \bar{c}\ 1.25 \times r_{Cl}$. In these bloods $r_{CO_2} = 1.7$ to $3 \times r_{Cl}$. If the intracellular CO_2 which is in excess of the amount to be expected from the chloride ratios is assumed to be "non-bicarbonate" CO_2, this "non-bicarbonate" CO_2 is found to comprise about two-thirds of the CO_2 in the red cell or about one-sixth of the CO_2 in the whole blood.

The influence of certain alcohols on the permeability of the erythrocyte. M. H. Jacobs and A. K. Parpart.

Low and moderate concentrations of the so-called indifferent narcotics have frequently been found to decrease cell permeability. Anselmino and Hoenig (*Pflügers. Arch.*, **225**: 56, 1930) have reported such an effect in the case of the penetration of human erythrocytes by several non-electrolytes including glycerol. The present study, involving the erythrocytes of a number of species of mammals and of some other vertebrates shows somewhat more complicated conditions. Thus, while n-butyl alcohol in concentrations from 0.0156 M to 0.25 M may greatly decrease the permeability of the erythrocytes, not only of man but also of the rat, rabbit, guinea pig, groundhog and several birds, the opposite effect is obtained with the erythrocytes of the ox, sheep, pig, horse, dog, cat and several reptiles and fishes. In general, these two groups of species are the same as those already distinguished by other properties of their erythrocytes (Jacobs, Glassman and Parpart, *Jour. Cell. Comp. Physiol.*, **7**: 197, 1935). In several cases involving decreased permeability, the order of effectiveness of a series of alcohols is: methyl < ethyl < propyl < butyl < amyl. With erythrocytes of the groundhog the effectiveness of *n*-butyl alcohol increases with increasing molecular weight of the penetrating substance in the order: ethylene glycol < glycerol < erythritol < mannitol. As contrasted with glycerol and related substances to which *n*-butyl alcohol increases the permeability in some species and decreases it in others, thiourea, under the

same conditions, always shows an increased permeability. The same was found to be true of lipoid-soluble substances such an monoacetin and ammonium salts of weak acids, which by hydrolysis give rise to NH_3 and lipoid-soluble acids. On the contrary, permeability to the ammonium salts of strong acids, where the penetration of the cell by ions is presumably involved, was in all species found to be greatly decreased.

Ionic exchanges of erythrocytes inferred from volume changes. A. K. Parpart, M. H. Jacobs and A. J. Dzienmian.

The exchange of $SO_4^=$ for Cl^- across the erythrocyte surface leads to a volume change of the erythrocyte which may be measured photoelectrically. Cells shrink when placed in isosmotic Na_2SO_4 solutions. The process is reversible since cells which have had the normal Cl^- of their interior replaced by $SO_4^=$ will swell when placed in isosmotic NaCl solutions. The exchange of Cl^- for $SO_4^=$ is relatively slow in comparison with the osmotic shifts that accompany it. The rate of this ionic exchange varies for the erythrocytes of different species of mammals in the order rat > guinea pig > rabbit > beef > pig, cat.

In view of the slowing effect that butyl alcohol, ammonium salicylate and ammonium benzoate have on hemolysis in ammonium chloride it appeared of interest to study the effect of these substances on the exchange of Cl^- for $SO_4^=$. In all of the species studied the above substances were found to slow the rate of this ionic exchange. Control experiments indicated that these substances, in the concentrations used, had little or no effect on the volume of erythrocytes suspended in isotonic NaCl.

Papers Read by Title

Oxygen as a controlling factor in the regeneration of Tubularia. L. G. Barth.

Preliminary experiments in which the distal end of the stem of *Tubularia* was inserted into a glass tube gave complete inhibition of regeneration of this end with a marked increase in rate at the opposite end. This indicated that low oxygen inhibited regeneration.

Following this the oxygen tension of sea water was varied and rate of regeneration was calculated by dividing the length of the primordia in micra by the number of hours necessary for its formation. Results of nine complete experiments agree in showing that from 4.0 to 21.6 cc. of oxygen per liter the rate of regeneration increased linearly with the logarithm of the oxygen tension. Below 3.0 cc. of oxygen per liter the rate of regeneration drops off to 0 at .35 cc. and the shape of the curve depends somewhat on the way in which the experiment is carried out. The lower limit for regeneration is between .35 cc. and 1 cc. of oxygen per liter. At .35 cc. complete inhibition results, which is reversible when stems are returned to high oxygen.

The results are interpreted as showing that regeneration of *Tubularia* is closely dependent on the amount of oxygen which the tissues receive. It is suggested that the perisarc of *Tubularia* is relatively impermable to oxygen and that the stimulus for regeneration is the admission of oxygen due to cutting of the perisarc.

The effects of different drugs on the melanophores of Fundulus heteroclitus. Sinisha B. Bogdanovitch.

In the following experiments the effects on melanophores of different drugs alone and in combination were studied. Isolated scales of *Fundulus heteroclitus*

were used and the drugs studied were atropine, pilocarpine, physostigmine, epinephrine, acetylcholine, mecholyl and deuterium oxide. The technical procedures were the same as those described in a previous paper. A summary of the results obtained follows:

Atropine: Expands melanophores and also does so after they had been previously contracted by epinephrine, deuterium oxide, acetylcholine or mecholyl. Ergotized melanophores were also expanded by atropine.

Epinephrine: Contracts melanophores and also does so after they had been previously expanded by pilocarpine, physostigmine or atropine. Ergotized melanophores are, as is well known, expanded by epinephrine.

Physostigmine: Expands melanophores and also does so after they had been previously contracted by epinephrine, acetylcholine, or mecholyl. Well ergotized melanophores are not expanded by physostigmine.

Pilocarpine behaves as physostigmine, but appears to be slightly more effective.

Acetylcholine contracts melanophores and also does so with melanophores previously expanded by physostigmine or pilocarpine. However, with melanophores previously expanded by atropine acetylcholine produces either very slight contractions or none at all. Ergotized melanophores are contracted by acetylcholine. Ergotized melanophores are, of course, already contracted, so that additional contraction is sometimes quite slight. Mecholyl behaves as acetylcholine but the effects appear to be more lasting.

Deuterium oxide contracts melanophores after they had been previously expanded by atropine, pilocarpine or physostigmine. Other effects of deuterium oxide on melanophores were described in a previous paper.

Scales removed from fish and kept in balanced solutions for two hours or more showed an initial expansion in epinephrine followed by contraction. Such a result, while possibly due to a pathological change, may explain contradictory results of previous investigators and offer a clue as to the nature of the innervation of melanophores.

Further investigations on the effect of tissue on different drugs. Sinisha B. Bogdanovitch.

In a former paper it was established that epinephrine and acetylcholine are destroyed by tissue (in this case scales of *Fundulus heteroclitus*), but that deuterium oxide protects these substances from such destruction. Using the same technique as in former experiments atropine, pilocarpine, physostigmine and mecholyl (acetyl β-methylcholine) were similarly investigated with the following results.

Solutions of atropine (sulphate) which had previously expanded melanophores, no longer did so or at most only slightly, after treatment with fish scales for 24 to 48 hours. However, atropine solutions to which scales and deuterium oxide were added for the same length of time, showed no change in expanding melanophores. Physostigmine (sulphate) and pilocarpine (chloride) solutions were not affected by fish scales even after 48 hours. Solutions of mecholyl (chloride) were also practically unchanged by similar treatment.

It thus appears that atropine is destroyed by fish scales as was epinephrine and acetylcholine, but that physostigmine, pilocarpine and mecholyl were not affected. In the former paper it was suggested that the destruction of epinephrine and acetylcholine by fish scales may be due to the action of enzymes such as acetylcholinase and some oxidase. In support of this view I observed that scales which had been heated at 100° C. for 10 minutes did not destroy atropine even after 48 hours. Similar experiments on epinephrine and acetylcholine gave the same results. This thermolability of the "destructive principle" in fish scales for these three substances is consistent with the enzyme hypothesis.

A quantitative study of the staining of marine eggs by neutral red. Barry Commoner.

Unfertilized eggs of *Chaetopterus* and *Nereis limbata* were stained in sea water containing various concentrations of neutral red at 14.0°, 15.5°, 17.0°, 18.4°, and 19.5° . The pH was maintained at 7.6.

Eggs were suspended in sea water acidified to pH 7.6. The density of eggs per ml. of suspension was determined and a known quantity of neutral red added. The same certified stain of 61 per cent dye content was used throughout. Concentrations are expressed as mgm. of this preparation.

Samples were removed periodically from the suspension and centrifuged to concentrate the eggs. The eggs were washed in sea water to remove unbound stain, again concentrated and shaken in 10 ml. of a neutral red extractant. (One volume of N/1 HCl and 9 volumes of 95 per cent ethyl alcohol.) The neutral red content of the egg sample was then determined by comparing (in a Duboscq colorimeter) the intensity of the extract with a standard neutral red solution. The neutral red content per unit volume of eggs was then calculated. (Maximum error; 3 per cent.)

Curves were plotted indicating the neutral red content of the eggs after various periods of time in the stain. Curves of similar shape were obtained for *Chaetopterus* and *Nereis* eggs. The maximum staining rate occurs during the first 20 minutes. Thereafter, the velocity of the process decreases until after 60–80 minutes an equilibrium is reached and no further change in neutral red content occurs.

At concentrations below .02 mgm./ml. the staining rate is proportional to the concentration of neutral red in the suspension. The stain content of the eggs at equilibrium is also proportional to the concentration of neutral red.

The initial staining velocity and the quantity of neutral red bound at equilibrium increase with temperature. Within the temperatures noted (concentration: .01 mgm./ml.) the Q_{10} obtained from the initial velocities of the staining curves of *Chaetopterus* eggs was 4.4.

The adaptation of Paramecium to sea water. John A. Frisch, S.J.

In cultures of hay and wheat infusions, in which the concentration of sea water was gradually increased by evaporation, many individuals survived and divided until a concentration between 35 and 40 per cent was reached; all died before a concentration of 45 per cent was reached. Daily observations of a culture in which the concentration of sea water increased to 40 per cent in 20 days showed that the average rate of pulsation and the average rate of feeding were lower day for day than in fresh water cultures; that both rates varied from day to day as in fresh water cultures, and that the rate of pulsation varied with the rate of feeding, increasing or decreasing from day to day, as the rate of feeding increased or decreased, just as in fresh water cultures. As the salt concentration increased the animals decreased in length and volume and became emaciated. Addition of nutrient medium or of bacteria to the cultures always resulted in an increase in the number and in the volume of the individuals, and in the rate of feeding and the rate of pulsation, except when the concentration of sea water had reached 40 per cent. The data indicate that the decrease in the rate of pulsation is not due to the increase in osmotic pressure of the medium, but to the decrease in the rate of feeding; that the decrease in the rate of feeding is due to a shortage of bacteria in the higher salt concentrations; that death is due to an increase in the viscosity of the protoplasm and to other toxic effects of the salts taken in by the cytostome, which result in the vacuolization of the protoplasm.

The water and fat content of skeletal muscle in marine fishes. Charlotte Haywood and Abby Turner.

In the course of a study of water balance in fishes of different life habits and phylogenetic relationships a series of determinations was made of both the water and fat content of skeletal muscle. This work was done in part at Bergens Museum Biologiske Stasjon, Herdla, Norway, and in part at the Marine Biological Laboratory, Woods Hole.

	No. of Individuals	Percentage of Fats	Percentage of Water in	
			Whole Muscle	Fat-free Muscle
Elasmobranchs:				
Mustelus canis	4	Trace	78.4	78.4
*Pristiurus catulus**	4	—	80.0	—
Galeus glaucus	1	Trace	81.0	81.0
Carcharias taurus	2	0	77.4	77.4
*Squalus acanthias**	1	—	78.4	—
*Squalus acanthias**	7	5.4	75.0	79.4
*Spinax niger**	4	—	80.6	—
Raja diaphanes	1	0	75.1	75.1
Raja stabuliforis	2	0	76.5	76.5
*Raja fallonica**	1	—	79.0	—
*Raja oxyrhynchus**	3	—	81.1	—
Narcacion nobilianus	3	0.4	83.9	84.2
Dasybatus marinus	2	Trace	77.9	77.9
*Chimaera monstrosa**	3	—	81.3	—
Teleosts:				
*Anguilla vulgaris**	3	—	66.4	—
Anguilla rostrata	1	4.7	73.3	77.0
*Scomber scombrus**	6	—	66.5	—
*Scomber scombrus**	8	5.4	70.5	74.5
Centropristes striatus	11	1.0	78.7	79.5
Cynoscion regalis	2	3.1	76.7	79.2
Tautoga onitis	4	Trace	79.8	79.8
*Cyclopterus lumpus**	1	—	72.2	—
Prionotus strigatus	2	0	79.9	79.9
Echeneis naucrates	1	3.7	73.1	76.2
Opsanus tau	2	Trace	80.2	80.2
*Anarhichas lupus**	6	—	84.0	—
*Gadus callarias**	8	—	80.3	—
*Gadus callarias**	6	0	81.7	81.7
*Gadus pollachius**	7	—	79.4	—
*Brosmius brosme**	6	—	80.0	—
*Molva molva**	2	—	80.3	—
*Lophius piscatorius**	5	—	84.3	—
*Lophius piscatorius**	2	0	87.1	87.1

* Determinations made in Norway.

Reactions to light of different intensities in Dolichoglossus kowalevskyi. Walter N. Hess.

For studying the sensitivity of *Dolichoglossus* to different light intensities a 100-watt Tungsten bulb, supplied with neutral tint Wratten filters, was used and

light of 115, 11.5, 1.15, and .115 m.c was thus obtained. For studying the relative photosensitivity of different regions of the body a fused quartz rod was suspended so that its base was illuminated by an arc lamp. The distal end, which was used in testing photosensitivity of small areas, was drawn out into a very small blunt point.

The animals reacted negatively to ordinary intensities of light but at .115 m.c. a rather large majority of positive responses occurred. By means of the pinpoint light it was shown that the animal is photosensitive over its entire body though certain regions are more light-sensitive than others. The tip of the proboscis is the most sensitive to light. In general, the dorsal and lateral body surfaces are more photosensitive than the ventral surface.

Mucous cells occur on the abdomen in patches interspersed with non-mucous cells. Very little response was obtained by illuminating the areas of mucous cells especially on the ventral surface, yet if the areas of non-mucous cells were illuminated the animals responded quickly.

These results show that the photoreceptor cells must be widely distributed and that they must be more numerous or more specialized in certain regions than in others. Cells of a certain type which correspond in their distribution to the relative photosensitivity of the animal have been identified. In keeping with the early chordate characteristics of this animal these cells resemble in their general morphology the retinal cells of vertebrates.

The hatching of the squid. Hope Hibbard.

The hatching gland of *Loligo pealii* is the so-called Hoyle's organ, a Y- or T-shaped gland which lies on the dorsal posterior surface of the larva, the arms of the gland extending out on the fins. It appears very early (about stage 12 of Naef in *L. vulgaris*), gradually matures, and disappears entirely shortly after the larva hatches. At the height of its development the gland protrudes slightly. A similar hatching gland has been described by Wintrebert and by Jung in several other cephalopods found in Europe. Staining the eggs or young embryos, even very heavily, with neutral red does not impede their normal development, and the organ in question can be very readily observed since it does not stain and appears as a white streak against a reddened mantle.

In early development the larva rotates inside its shell by ciliary action, pushing the posterior end up until gravity causes it to lose balance, and fall to the lower side of the shell whence rotation recommences. But as hatching approaches the animal adheres very firmly to the shell as if stuck by a secretion, in a disc-shaped area around the hatching gland. The head is directed downward, and rhythmic contractions of the free part of the mantle are constant. The gland itself appears to wriggle and squirm from time to time, due to underlying muscle cells, pushing ever closer to the shell. In some cases the weak place bulges considerably before giving way. Finally the shell appears to dissolve away and the animal swims out backward through a neat round hole. There is no tearing, and after the larva has emerged the round hole remains in the empty shell.

The cells of the gland are very long, slender ones with the nucleus at the proximal end deeply embedded in the mantle. The secretion is conspicuously granular and resembles well-fixed zymogen granules in a pancreas cell. Pressure on the cells forces the granules out, and they maintain their individuality outside. After hatching, the cells remain for a few hours, but the distinctness of the unsecreted granules disappears, the material becomes more fluid and runs together. Further cytological examination of the evolution of these cells is under way.

The vacuole system of the marine Amoeba, Flabellula mira. D. L. Hopkins.

In this study the vacuole system was observed when the amoebae were under normal conditions; slightly abnormal conditions; unstained; stained with the vital dyes, neutral red, Nile blue sulfate and Janus green B; and after impregnation with osmium tetroxide.

Mitochondria which stain with Janus green B are not present in normal fully active amoebae, but appear and may be stained with Janus green B when the amoebae are placed under slightly abnormal conditions. The mitochondrial substance in fully active amoebae appears to be contained in very small vacuoles. These vacuoles arise de novo from the protoplasm, grow, fuse with each other and engulf food. The food vacuoles and the vacuoles containing no food fuse with each other, forming larger and larger vacuoles, until finally a large fusion vacuole, the *cloacal vacuole,* is formed. The fluid and solid contents of this cloacal vacuole after a period of digestion are voided to the outside.

Nile blue sulfate stains all these vacuoles blue from the time of their origin until after their entrance into the cloacal vacuole where the blue fades out, in some cases becoming clear of color directly, while in others it becomes pink or red and then clear. This indicates that fatty acids are present in all vacuoles, and perhaps some neutral fat. Both of these types of substances disappear from the vacuoles before the residue is voided.

The substance stained by neutral red is present in all the smaller vacuoles but disappears entirely before they enter into the cloacal vacuole.

Neutral red staining, and osmium tetroxide impregnation of amoebae previously treated with neutral red show that the smaller vacuoles and the cloacal vacuoles are in a more highly oxidized, or in other words, in a less reduced condition than are the vacuoles of intermediate size.

Effect of electrical shocks upon the division rate of Stylonychia pustulata as measured by the interdivisional period. Lois Hutchings.

The normal interdivisional period of *Stylonychia pustulata* in summer was found to be six to ten hours. Most frequently the control animals underwent division once every eight hours. Every animal used in these experiments came from one protozoön. Isolation dishes were used. The culture medium was filtered hay-tea in which mass cultures had lived previously for five to twenty days, average pH 7.4. A piece of dry outmeal, one-millimeter square, was placed in each dish when the medium was changed each day.

Two new dry-cell batteries were connected in series to the primary circuit of an inductorium, Harvard type. The secondary coil was kept at the eight-centimeter mark. The current which flowed through the secondary circuit had a non-smooth alternation of 106 times a second, known as tetanizing current. A foot switch closed the circuit at will. From the secondary circuit were two insulated copper wires to whose tips were soldered one-inch lengths of No. 30 platinum wire. Each point electrode was so manipulated by hand that a constant distance of twice the body-length between it and the animal was maintained. Even when the protozoön moved it was usually possible to keep the interelectrodal distance constant.

Each *Stylonychia* received treatment hourly. For several reasons physiological indices of sufficient treatment were adopted. In order of importance these were: degree of swelling, loss of lateral orientation to the current, and rapid spinning. The length of treatment varied from 10–75 seconds.

Disregarding the five cases in which the treated *Stylonychia* had the same length of interdivisional period as the control animals, the results may be simply stated. Fifteen treated animals underwent an average shortening of the inter-

divisional period by 2 hours, but 102 animals had an average lengthening of the interdivisional period by 6¼ hours. The range of shortening was ½-3½ hours and the range of lengthening was ½–23 hours. In other words, although in 13 per cent of the cases application of electrical shocks caused an apparent acceleration of the division rate, in far the greater number of cases, 87 per cent, such shocks caused a slowing down of the division rate.

Further comparative studies on the permeability of the erythrocyte. M. H. Jacobs and H. N. Glassman.

In earlier papers (*Proc. Am. Philos. Soc. 70,* 363, 1931; *Jour. Cell. Compar. Physiol. 7,* 197, 1935) certain characteristic differences in the permeability of the erythrocytes of different species of vertebrates to dissolved substances were reported. The results previously obtained by means of the hemolysis method were not very satisfactory in the case of many species having nucleated erythrocytes because of the failure of suspensions of the latter to become completely transparent on hemolysis. By taking advantage of the fact that this difficulty is largely removed by the addition to the hemolytic solutions of 0.001 M $NaHCO_3$, without greatly altering the time of hemolysis in the case of most of the species studied, further data have been obtained on the permeability to ethylene glycol, glycerol, urea, and thiourea, of the erythrocytes of 9 species of elosmobranchs, 14 of teleosts, 2 of frogs, 6 of turtles, 4 of snakes and 4 of birds. In addition to various highly characteristic specific differences the following general peculiarities of the larger groups—subject to some exceptions which cannot here be mentioned—are of interest. *Fishes:* permeability to ethylene glycol greatest; that to urea and glycerol highly variable from species to species; permeability to thiourea usually greater than that to urea. *Amphibia:* (data as yet too scanty to permit generalizations). *Birds* (gull, tern, English sparrow, pigeon): permeability to ethylene glycol and glycerol both very great and nearly equal; that to thiourea much less and to urea least of all. Earlier experiments showed similar conditions in the starling but not in the duck and the chicken. *Reptiles:* permeability to urea relatively great, followed by that to ethylene glycol and at a much greater distance by that to thiourea. Permeability to glycerol slight as compared with that to the other substances. *Mammals:* (for comparison) permeability to urea extremely great; that to ethylene glycol much less and to thiourea still less; permeability to glycerol in some species greater and in some species less than that to thiourea.

The attenuation of toxins by interfacial adsorption. J. M. Johlin.

In view of the fact that some toxins, upon standing, are changed into toxoids, the writer thought it likely that similar attenuations might be brought about more speedily by methods which induce interfacial adsorption, and has applied such a method to the attenuation of ricin, tetanus toxin and snake venom. This speedy attenuation may be regarded as the result of the catalytic influence of the interface in bringing about an increased surface concentration of properly oriented molecules of the toxin at the interface. Adsorption was brought about by emulsification of the toxin with a volatile inert liquid such as ether or chloroform which could be easily removed by evaporation under reduced pressure afterwards. Ricin, thus attenuated, was found to be ten thousand times less active when injected intracutaneously into rabbits. One thousand M.L.D.'s of tetanus toxin, when attenuated, could be injected subcutaneously into mice repeatedly at two-day intervals without killing them or causing any apparent prolonged discomfort. Multiple lethal doses of mocassin and rattlesnake venom could also be injected into mice without causing death or producing the usual signs of damage caused by the untreated venoms. In such immunization experiments with rabbits as

have been carried out it was found that the attenuated toxin had retained its antigenic property and could be injected daily in large amounts without producing visible symptoms of any kind.

The action of acetylcholine on the skeletal muscle fibers of the frog. Elsa M. Keil and F. J. M. Sichel.

In a previous report by the present authors (*Biol. Bull.* **71**: 402, 1936) it was shown that the application or injection of very small amounts (5000 μ^3) of acetylcholine in concentrations ranging from 1 in 10^8 to 1 in 10^{11} had no effect on the isolated single fiber (adductor muscle of frog's leg, *Rana pipiens*), beyond the effects attributable to the medium in which the acetylcholine was dissolved. Recent experiments with acetylcholine on single muscle fibers isolated from the sartorius of the frog confirm this. However, if the sartorius be dissected from the frog, and even if both the tibial and pelvic ends be cut across, then acetylcholine will cause a propagated twitch when applied as a small droplet with a micropipette to the dorsal surface of the muscle in the region of the nerve twigs. This twitch is noticed in only those muscle fiber bundles which are in the vicinity of the point of application of the acetylcholine. If either the pelvic or tibial half of the sartorius is split lengthwise into two parts, one containing about twenty fibers, the other the rest of the fibers, then acetylcholine applied to the surface of either of these bundles also causes a similar propagated twitch response. If the smaller bundle of fibers is progressively split into still smaller groups, a degree of subdivision will eventually be reached such that even dilute acetylcholine will no longer evoke twitches in fibers still irritable to electrical stimulation. This apparently occurs when the subdivision has interfered with the nerve supply, the fine nerve twigs being injured by the process of dissection.

We conclude that these experiments offer further evidence in support of our previous statement that the action of acetylcholine is not upon the contractile mechanism of the muscle directly. We further conclude that acetylcholine evokes twitches in the sartorius muscle of the frog only when the terminal nerve supply or possibly some junctional tissue or receptor is intact. These conclusions are in accord with Garrey's experiments on turtle and *Limulus* heart, and with Armstrong's experiments on embryonic *Fundulus* heart.

The effect of pituitary on nuclear changes in the egg of the frog. John A. Moore.

In an effort to separate the processes of maturation and ovulation in the frog, *Rana pipiens*, mature females were given homoplastic anterior lobe injections during the middle of the summer, at which time it has not been possible to stimulate the release of eggs. Parts of the ovary were removed before and after injection so the same animal serves as a control and as an experimental. It was found that by thirty to forty hours after injection (at 25–27° C.) the germinal vesicle had moved from its position in the center of the egg to the surface at the animal pole. This movement is found only in the large eggs with evenly distributed yolk. By one hundred and twenty hours the eggs are degenerating and have been heavily invaded by phagocytes. This latter condition is similar to that found by Miss King in the few mature eggs that remained in the ovary of the toad after the breeding season.

The effect of urea upon the surface of unfertilized Arbacia punctulata eggs. Floyd Moser.

Following the procedure of Moore (*Protoplasma*, 1930) *Arbacia punctulata* eggs were treated with molar urea solutions. After a total exposure of no

more than two minutes, the eggs were transferred to sea water. Some of them were then inseminated and others served as uninseminated controls.

Within three or four hours irregularly cleaving eggs (two to eight cells) may be found among both the treated inseminated eggs and the treated uninseminated eggs. At the same time apparently normal blastulae with membranes perhaps a little thinner than normal may be found among the treated inseminated eggs, but not among the uninseminated eggs. In some cases it is possible to demonstrate very thin and but slightly elevated membranes in these irregularly cleaving eggs.

If a small drop of sea water containing centrifuged or uncentrifuged eggs be placed on a slide and a larger drop of molar urea solution be caused to flow into the drop of eggs while the latter are being observed under the high power of the microscope, a breakdown of cortical granules occurs. Immediately following this cortical response membrane elevation occurs with the formation of a very wide perivitelline space. Within a few seconds the elevated membrane begins to recede toward the egg surface, becoming thinner as it moves, until finally, in some cases, no vestige of the membrane remains. The cortical response secured in these urea-treated eggs is essentially like that obtained upon stimulation with sperm cells or with agents which induce parthenogenesis such as saponin. Similar cortical responses are secured in molar thiourea and molar glycerine solutions.

It is evident, therefore, that these non-electrolyte solutions do not prevent membrane elevation in *Arbacia punctulata* eggs (either irreversibly or otherwise), but may actually stimulate the egg to cortical response and subsequent membrane elevation. Indeed, delayed and irregular cleavage often follows the cortical response after treatment with these agents.

The cortical response of Arbacia punctulata eggs to direct current. Floyd Moser.

In a previous abstract (*Biological Bulletin*, 1935) it was noted that a layer of granules located in the cortex of the unfertilized *Arbacia* egg breaks down when the egg is subjected to a number of different stimulating agents, as well as to normal insemination. The present experiments with direct current extend the list of stimulating agents reported earlier.

A non-polarizable system of Cu-$CuSO_4$ electrodes with agar bridges was used. The eggs were placed in small glass tubes both ends of which were plugged with 2 per cent agar made up in sea water. The current intensity varied from 1.5 to 20.0 milliamperes.

As soon as the circuit is closed the eggs begin to move toward the anode. Within a few seconds cortical layer granules on the anodal side of the egg break down, releasing the fertilization membrane from that portion of the egg over which the response has taken place. When the current is reversed the same response takes place on the opposite side of the egg. Generally some of the cytoplasmic granules beneath the cortex also exhibit this breakdown phenomenon. Indeed, when the current flows for too long a time the cortical response is followed or accompanied by complete cytolysis.

In the uncentrifuged eggs the cortical response stops when the circuit is broken, but generally completes itself in wave-like fashion in the centrifuged egg. The so-called fifth layer usually breaks down in the centrifuged eggs.

If the eggs are treated with M/4 ammonium oxalate, 0.3 M potassium oxalate or 0.35 M potassium citrate there is no granule breakdown or membrane elevation upon exposure to direct current.

The observations here recorded are in general agreement with the point of view developed by Heilbrunn and his students in their studies of stimulation.

The histology of the retractor muscles of Thyone briareus Lesueur. Magnus Olson.

A decided gap exists in our knowledge of the comparative histology of invertebrate muscle. This fact has made desirable a more comprehensive study of invertebrate muscles particularly since a considerable number of these have been employed in recent physiological research.

The only extensive paper on the histology of holothurian muscle (Hall, 1927 on *Cucumaria*) misinterprets their essential structure and has been responsible for some erroneous conclusions by workers in muscle physiology.

The present study has shown that the retractor muscles of *Thyone* consist of enormously long narrow unstriated fibers imbedded in a connective tissue network. The fibers occur in bundles of 2–15 and in each bundle are arranged circularly in a single layer. Each bundle is surrounded by a comparatively dense layer of connective tissue which penetrates only sparingly into the interior of the bundle. Hall interprets the bundles of fibers as being single cells each of which contains many enormously large fibrils. That these so-called fibrils of Hall are actually the muscle fibers can be determined readily not only by the presence of connective tissue within the fiber bundles but also by a study of the arrangement of fiber nuclei. Critical examination of this arrangement reveals a peculiar picture. In the contracted fiber the nucleus lies on the outside of the fiber and may be connected to it only by a narrow protoplasmic strand. In the extended condition of the fiber the nucleus is elongated in the axis of the fiber and lies closely pressed against it. Occasionally nuclei are found directly within the fibers. As far as it has been possible to determine, the fibers appear to be uninucleate. Connective tissue nuclei occur not infrequently within the bundles of fibers.

The fibers are extremely extensible. The muscles may vary in length from about 5 mm. in the contracted condition to 5 or 6 cm. in normal extension or after anaesthetization with magnesium chloride. The fiber diameter varies from 5–10 mu in a contracted condition to 2–4 mu in an extended condition.

From teased preparations it may be seen that although the fibers are extremely long, they do not extend the full length of the muscle. The terminal portions of the fibers are long and tapering. The fibers are spherical or hemispherical in cross-section in a contracted condition, laterally compressed in an extended condition. The connective tissue fibers appear to run at right angles to the muscle fibers and form a dense network at the periphery of the muscle.

Strength-duration curves of nerve fibers in the squid. C. Ladd Prosser and A. H. Chambers, Jr.

Giant nerve fibers and fin nerve fibers of the squid were stimulated by condenser discharges through calomel electrodes and strength-duration curves were obtained. Excitation constants (Hill's characteristic time, k) range from .33 to .60 milliseconds and average .47 milliseconds for the isolated giant fibers. The time constants are essentially the same (characteristic time averages .42 milliseconds) for the giant fiber intact in the stellar nerve as for the isolated fiber, but the whole strength-duration curve is shifted upward with the intact nerve. Hence the effect of other fibers in a nerve trunk upon the excitation of one fiber is to serve as a shunt for the stimulating current.

Decreasing the electrode separation from 12 to 3 mm. shifts the strength-duration curve diagonally upward and to the left, with the result that the time constants are shorter at the smaller separation. As the preparation deteriorates the threshold rises and the characteristic times become shorter, i.e. the change in the strength-duration curve is similar to the change resulting from shortening in-

terelectrode distance. With electrodes 7 mm. in diameter the time constants are approximately 1.5 times as long as with electrodes less than 0.6 mm.

The diameters of the giant nerve fibers are approximately 100 times those of the fin nerve fibers. The fin nerve fibers have characteristic times approximately twice as long as the giant fibers (.88 millisecond with electrode separation of 12–14 mm.).

New structures induced by implants of adult nerve cord in the polychaete, Clymenella torquata. Leonard P. Sayles.

For several summers a study has been made in which a piece of adult nerve cord, removed from one *Clymenella*, was transplanted into another. All implants were of one to two segments in length. They were placed in the body wall in a dorso-lateral region to minimize the chance of injuring the nerve cord of the host. No bud has been formed in any case in which the nerve cord slipped into the coelom free from the body wall.

To date 63 buds have been induced by this type of implant. These include 12 heads, 29 tails, 2 double buds each consisting of a head and a tail, and 20 irregular or weakly developed buds. No head buds have appeared posterior to the tenth segment of the host. One of the double structures was formed at the fourth, the other at the eighth.

Both the source of the implant and the region of implantation in the host seem to influence the type of bud to be formed. Many more cases must be secured before any conclusions can be drawn concerning this point. The possibility that orientation of the implant may also be a factor has not been fully eliminated.

Cytoplasmic division in type B of the giant amoeba Chaos chaos Linnaeus. A. A. Schaeffer.

Although type A and type B of *Chaos chaos* are very closely similar, morphologically, to the common laboratory amoeba, *Chaos diffluens*, there are certain characteristics which set them apart very definitely. One of these characteristics is the striking phenomenon of frequent division into three daughters instead of two, which occurs in both types of *chaos*. During division the amoebas of type A round up into a mulberry-like shape, while those of type B, which are also berry-shaped at first, soon flatten out somewhat, so that one cen predict to a certain extent the number of daughters about to be produced, by counting the well-defined lobes of the flattened-out amoeba. The number of daughters may be 0, 2, 3, 4, 5, or 6, and the number of lobes formed before separation is well correlated with the number of daughters produced. The amount of undigested food in the amoeba influences the number of daughters to be formed, to a markedly greater extent than it influences the number of lobes formed during division. In both cases the number is reduced by large quantities of undigested food.

In type A, more divisions result in 3 daughters than in all the other classes put together, while in type B, in a culture with food in excess of needs, as many amoebas divide into 2 as into 3. But the number of amoebas showing 3 lobes prior to division, under these cultural conditions, is much greater than those with 0, 2 and 4 lobes combined. The average variation in size between twins from a 3-lobed parent is also greater than that between twins or triplets whose parents showed 2 and 3 lobes respectively before separation of daughters. More sextuplets occur than would be expected on pure chance, although not enough data are at hand to warrant the conclusion that the curve of frequency rises again to some degree at 6 daughters.

Something akin to a trigonal field of force in an apparently turbulent medium exists here which stands in striking contrast to the common halving type of cytoplasmic division.

The sense of taste in the free fin rays of the sea robin (Prionotus). Ernst Scharrer.

Experiments were carried on to show that the free fin rays of the sea robin are chemoreceptors as well as organs of locomotion. Blinded sea robins react after 3–4 weeks of acclimatisation in the aquarium with snapping reactions when the juice of a spider crab or of a clam is gently poured on the free fin rays by means of a pipette. A water current caused by a pipette never is followed by a reaction. The behavior of the sea robin is therefore identical with that of the Mediterranean genus *Trigla* (Scharrer, 1935) and it might be concluded that all Triglidae use the three free rays of the pectoral fins as chemoreceptors in search for food.

Calcium and magnesium in relation to longevity of Mactra, Nereis and Hydroides egg cells. Victor Schechter.

Experiments on the relation of calcium to longevity of unfertilized *Arbacia* egg cells (*Biol. Bull.*, **72**: 366) were extended to the cells of the mollusk, *Mactra*, and to those of *Nereis* and *Hydroides*. The *Mactra* egg, which in change in rate of hypotonic cytolysis with age (*Biol. Bull.*, **71**: 410) follows the *Arbacia* egg closely, also exhibits prolongation of life in low calcium most clearly. This action, as in *Arbacia*, seems to be one rather specific to calcium as a decrease in magnesium alone does not produce it nor does lowering of both magnesium and calcium have an added effect. Increase of magnesium above the normal concentration in sea water did result in an additive effect on longevity.

The two phenomena need not, however, be related. The first sign of deterioration in the *Mactra* egg is usually the breakdown of the germinal vesicle. Insemination also causes germinal vesicle breakdown. In eggs which cannot be activated by sperm, cytoplasmic cytolysis may occur with age while the germinal vesicle is still intact. With such eggs, increasing the magnesium concentration retarded cytoplasmic disintegration; and the eggs then lived longest if calcium was low.

Therefore, in so far as the phenomena of aging may be partitioned between the various cellular components, the effect of low calcium is tentatively regarded as one which retards deteriorative changes in the nucleus, whereas the action of high magnesium may be primarily in the nature of an anaesthesia. Magnesium may act chiefly on the cell membrane since with increased magnesium the egg cells retain their spherical shape beyond the time when controls appear plasmolyzed.

Preliminary experiments with *Hydroides* eggs show prolongation with low calcium.

In one-sixth the normal calcium concentration the reaction of *Nereis* eggs was variable. This is possibly due to egg condition and it may be that with different calcium and magnesium concentrations prolongation of life in these cells will also be obtained consistently.

A convenient method for the measurement of nerve respiration. Francis O. Schmitt and Otto H. Schmitt.

For the determination of the effect of electrical stimulation on the oxygen consumption of single nerves it is desirable that readings be made over short intervals of time (every 2–3 minutes) and that the variations between individual readings be small (ca 2–5 per cent). Even with differential volumeters of small volume this requires that temperature fluctuations between the two vessels be reduced to a minimum. This may be accomplished without the use of a precision thermostat by immersing the vessel in a mercury bath, the latter in turn being enclosed in a well insulated box. The mercury is contained in a copper box lined inside and outside with insulating material and provided with a glass window which

permits following the movement of the index droplet with a traveling microscope, the capillary being only partially immersed in mercury and bent so that the vessels are submerged well below the surface of the mercury. Wires from the electrodes are brought out through rubber tubes. The capillary is illuminated by filtered light brought in by a glass rod fastened on the carriage of the travelling microscope. The copper box is surrounded by kapok or eiderdown contained in a well-insulated box which also carries the traveling microscope. The entire unit is easily portable and, at least for work at room temperature, the method has been found quite as satisfactory as the conventional method which requires a precision thermostat of special design.

Temporal relations in the excitation of the isolated muscle fiber. F. J. M. Sichel and C. Ladd Prosser.

The possibility of spatial summation in the excitation of the isolated muscle fiber has been reported previously (Sichel and Prosser, *Biol. Bull.*, **69**: 343, 1935). In the present case the excitatory effects of two stimuli separated by a varying interval have been studied. The stimuli were obtained from two condensers, each controlled by a gas triode, discharging into a common resistance. The interval between the two shocks was varied by a Lucas' spring rheotome in the grid circuits of the gas triodes. The time-constant for the discharge of each condenser was 1 millisecond.

The fiber (adductor longus, *Rana catesbiana*) was isolated and mounted in a manner previously described (Sichel, *Jour. Cell. Compar. Physiol.* **5**: 21, 1934) and the isometric tension recorded by a micro-lever (Brown and Sichel, *Jour. Cell. Compar. Physiol.* **8**: 315, 1936). The electrodes were silver-silver chloride, and were sufficiently large to make the spreading of the field at the ends of the fiber unimportant.

The tension developed falls continuously as the interval between the shocks increases, rapidly at first, then more and more slowly toward a steady value reached when the interval between shocks is about 20 milliseconds. The tension is then one-third to three-quarters of the tension developed when the stimuli are simultaneous.

If a similar experiment is done on the whole sartorius using maximal stimuli, as the interval between stimuli increases the tension at first remains constant, this being related to the absolute refractory period, then increases rather abruptly to a new level. With submaximal stimuli there is an initial fall to the constant level followed by a subsequent rise.

The absence in the case of the isolated fibers of the low constant level of tension for short intervals, and the smooth nature of the tension-interval curve we attribute to the absence of an absolute refractory period in the excitation of these fibers.

Electrolytes in Phascolosoma muscle. H. Burr Steinbach.

The retractor muscles of the marine annelid *Phascolosoma gouldi* consist of closely packed long smooth muscle fibers. Muscles freshly excised from the animal contain the following average amounts of inorganic elements, expressed as milliequivalents per hundred grams wet weight: Na 10.4, Cl 9.1, K 10.6, Ca 0.85. The body fluid surrounding the muscles contains approximately: Na 38, Cl 43, K 3.5, Ca 2.0. On immersion for two hours or more in sea water, the tissues gain weight by 11 per cent, chloride increases by 16 per cent, Na by 14 per cent, Ca by 13 per cent while 11 per cent of the K is lost. These changes can be partly accounted for by assuming an extra-cellular chloride space which alone is involved in the tissue swelling and the substitution of sea water for body fluid. Both Cl and Ca increase somewhat more than would be expected on this simple hypothesis.

The Na increase is less than would be expected while a large part of the K loss must involve the fibers themselves.

Muscles were soaked for two hours or more in artificial sea water containing known concentrations of K or Ca, or in sea water diluted with isotonic sucrose solution.

Na, Cl and Ca contents of the muscle bear a simple linear relationship to the concentrations of these ions in the immersion fluid. All of the Na and Cl appears perfectly free and diffusible, the slope of the curves Na-tissue/Na-fluid and Cl-tissue/Cl-fluid indicating about 30 per cent chloride (extra cellular) space at all concentrations. About half of the initial Ca content does not diffuse into Ca-free sea water or isotonic sucrose, but using that figure as zero concentration of Ca in the muscle, the curve for Ca-tissue/Ca-fluid is linear and has a slope indicating about 40 per cent diffusion space. Eighty per cent of the K does not diffuse into a K-free medium. As the K content of the fluid is increased the K of the muscle increases, rapidly at first then more slowly until finally a linear relationship is shown between K-tissue and K-fluid at concentrations between 0.05 M and 0.25 M K in the fluid.

Leaf development and vegetative propagation in Polystichum plaschnickianum. Harry N. Stoudt.

Material for this work was collected by the writer in the rain forests along the Vinegar Hill Trail, Jamaica, B. W. I. in the summer of 1936 and the investigation was undertaken at the suggestion of the late Professor Duncan S. Johnson.

Leaves of this plant are produced from segments of the apical cell of the rhizome. The leaf grows, apparently, by means of a bifacial initial which persists in the mature leaf. This cell continues to cut off segments to form the first embryonic leaf of the plantlet at the apex of the leaf. An apical cell is cut out from a marginal cell of one of the last segments of the parent leaf to form the shoot of the plantlet. Later leaves are formed from segments cut off from this cell. A meristematic cushion forms at the apex of the parent leaf and adventitious roots arise endogenously in this region and grow by means of a tetrahedral initial. By this time the roots penetrate the moist soil and the plantlet matures. Simultaneous with plantlet development the parent leaf develops wings that encircle the plantlet. Further investigation is needed to understand the origin and development of these wings. When several leaves and roots have formed the plantlet becomes independent and this region of the parent leaf decays. The remaining portion of the mother leaf persists to carry on spore formation.

Morphological and experimental cytology of lobster spermatozoa. (Preliminary note.) T. Terni.

In Woods Hole I have begun to microdissect the spermatozoa of decapods, most appropriate material because of its immobility and large size. I refer here only to my preliminary results.

1. The three long rays of the head have a permanent shape which is slightly curved. They are elastic, and if deformed by the needles, they immediately resume their original form. The rays are, on the other hand, neither plastic nor extensible if pulled with the needle. The mechanical stimulation does not induce visible contraction of the ray-like filament. Movements of these, if really existing, are in all cases so slow that only the microcinematographical technique will reveal them.

2. If with the tip of the needle one exerts considerable pressure, although not sufficient to injure, on the anterior part of the spermatozoa, there always appears the phenomenon of the so-called "explosion" (Koltzoff). The process seems to be irreversible; I have followed for 20 hours the fate of the exploded spermatozoa without changes. In order that the explosion should take place after pricking, it is necessary that the spermatozoa be freshly removed from the spermaduct.

3. In the posterior part of the small tube contained in the chitin capsule of the tail there exists before the explosion material strongly acidophilous. After the explosion the capsule and its content become suddenly basophilous while the nuclear chromatin loses its strong basophily, perhaps because it diffuses in the capsule.

4. With microdissection it is easy to follow the very rapid process of invagination of the anterior part of the spermatozoön into the capsule, which simultaneously becomes swollen. The examination of good preparations (ac. osmic-iron hemat.) shows the fact that the basis of the cephalic rays are pulled into the tail portion like an umbrella that closes itself into a sheath. Simultaneously from the posterior pore of the tube there flows out a basophilous body, perhaps the anterior centrosome.

Observations on arterial pressure in marine fishes. Abby H. Turner and Charlotte Haywood.

In an effort to determine representative values for the arterial pressure supplying the organs of fishes the anterior mesenteric artery was employed for cannulation. Pressures were measured in centimeters of isotonic saline, using a manometer. Respiration was well maintained by a stream of water over the gills, but obvious disadvantages in the method are that the fish is not entirely submerged in water and that the body cavity must be opened.

A number of the determinations were made in the summer of 1936 at Bergens Museum Biologiske Stasjon, Herdla, Norway.

	No. of Individuals	Range of Values in cm. Saline	Average Values in cm. Saline
Elasmobranchs:			
Mustelus canis	4	11. –25.5	16.7
Carcharias taurus	2	16. –19.	17.5
Squalus acanthias	7	8.5–18.8	15.1
Raja diaphanes	1		22.
Raja stabuliforis	2	12.5–21.5	17.
Raja oxyrhynchus	1		8.3
Narcacion nobilianus	3	Extremely low	
Dasybatus marinus	2	9. –10.1	9.5
Teleosts:			
Centropristes striatus	1		37.
Anarhichas lupus	4	9.7–15.	11.3
Gadus callarias	12	10.9–43.	27.7
Gadus pollachius	2	12.2; 12.2	12.2
Lophius piscatorius	6	9. –23.7	16.7

Respiratory rate and length of fertilizable life of unfertilized Arbacia eggs under sterile and non-sterile conditions. Albert Tyler, Nelda Ricci and N. H. Horowitz.

The respiratory rate of unfertilized eggs of marine animals does not remain constant with time, but sooner or later shows a marked rise. This rise occurs at the time when the eggs lose their fertilizability (Tyler and Humason, 1937). Measurements of the respiration of *Arbacia* eggs in 2 per cent alcohol or other agents that are known to prolong the fertilizable life (Whitaker, 1936) show that the rise is correspondingly delayed, while the initial absolute rate is unaffected.

By plating out for marine bacteria from the respiration vessels, it is found that the bacteria increase as the rate of respiration rises both in sea water and in alcohol. When sterile or almost sterile cultures of eggs are run it is seen that the respiratory rate remains low and shows even a slight drop. Such eggs have been run for four days without showing a rise in respiratory rate, whereas under ordinary conditions the rise begins at about 10 hours.

Culturing eggs under sterile conditions in sealed dishes prolongs their fertilizable life as Gorham and Tower (1902) found, some of the eggs lasting as long as 20 days. Under such conditions alcohol appears to shorten the fertilizable life. Addition of bacteria accelerates somewhat the disintegration of the eggs. Alcohol in ordinary sea water or in the presence of small amounts of bacteria considerably prolongs the life of the egg. In the presence of large quantities of bacteria, 2 per cent alcohol is toxic within a few hours, such cultures showing a drop in pH to as low as 4.60.

The results evidently mean that bacteria contribute to the destruction of the eggs only after certain disintegrative changes have occurred. The agents that prolong the life do not act bactericidally, but by retarding these initial spontaneous changes on the part of the egg.

The effect upon gastrulation and differentiation in Arbacia of $NiCl_2$, $CuCl_2$, and Na_2SiO_3 in modified artificial sea water. A. J. Waterman.

A formula for artificial sea water consisting of 390 cc. 1m NaCl, 9 cc. 1m KCl, 23.35 cc. 1m $MgCl_2$, 25.5 cc. 1m $MgSO_4$, 9.35 cc. 1m $CaCl_2$, about 0.1 gram $NaHCO_3$, additional water to make one liter (Chambers), and corrected for pH supports development of *Arbacia* from the blastula stage in a typical manner. Modifications and omissions of salt proportions have been studied in relation to effects on gastrulation and embryonic differentiation (Runnstrom, Lindahl, Dalcq). The addition of $NiCl_2$ and $CuCl_2$ to the above has been studied and comparison made with previous observations with these heavy metals in sea water. Increase, decrease or omission of Mg, SO_4, K, and Ca influence type and rate of effect of the metals. Exogastrulation occurred in some experiments and gastrulation could be temporarily inhibited. The blastula stage was used in all experiments.

Na_2SiO_3 was tested in relation to respiratory and cell division rates of the embryos in sea water and in the above modifications. In relatively large concentrations silicon is non-toxic and does not affect gastrulation. Normal cleavage rate is increased, top-swimming blastulae appear earlier, plutei mature sooner, and gastrulation may be accelerated. There is no noticeable differential acceleration of germ-layer differentiation. This has also been studied in artificial media where the salt balance has been disturbed or metallic salts added. Toxicity may be increased. Addition of silicon (Mast and Pace) to cultures lacking sulphur may or may not influence developmental rate.

Compounds of silicon are very abundant in nature and present in all waters (Reynolds, 1909). Both animals and plants use silicon and it is found in animal tissues. According to Mast and Pace, 1937, it increases rates of respiration and division in *Chilomonas* and has a catalytic action on synthesis of complex organic compounds.

Studies on living conjugants of Paramecium caudatum. Ralph Wichterman.

A new method of approach to a better understanding of the problem of sexuality in *Paramecium* is presented. By means of a precision micro-compressor, it

is possible to observe in the living condition, the behavor of nuclear phenomena and establish time relationships during conjugation.

With a micro-pipette, a single pair of recently joined conjugants is placed on the glass slide of the compressor in a small drop of the culture medium and studied at 26° C. The metal top holding the glass cover-slip is screwed down while observations continue with the microscope. The animals are prevented from spiraling between the two pieces of glass and allowed to move around slowly. Adjustments within a few microns make this possible.

The divisions of the micronuclei and their behavior have been seen and photographed in the living condition. The micronucleus of each conjugant is seen to leave its place near the macronucleus and increase in size. While this enlargement takes approximately four hours, the end of the division (including the anaphase and telophase stages) is more rapid requiring only about 18 minutes. The "crescent" prophase stage and long anaphasic-telophasic separation spindle so characteristic of this division are clearly shown. The swelling at the center of the separation spindle becomes separated and is passed into the cytoplasm. Cyclosis moves it about until it ultimately degenerates. Each product of the first division enters into the second where again two long spindles in each conjugant are visible. The second division requires 50 minutes for completion from the time the first division products are formed. The anaphase and telophase stages are still more rapid, requiring only nine minutes. Degeneration of three of the four products of the second division is observed. Behavior of the pronuclei after the third division is being more carefully studied in an effort to record their movements accurately. The pronuclei of a given conjugant have been seen to fuse and form a synkaryon in the same individual. In a number of observations, definite evidence has been obtained to show that crossing-over of pronuclei does not occur. Too, no evidence has been obtained to show that crossing-over of pronuclei takes place.

In view of these observations, any conclusions, genetical or otherwise, based on the assumption that there is crossing-over of pronuclei, are open to grave question.

Photographs are being made of the living conjugants and will be included in the full paper. It is hoped also to make a motion picture of the entire process.

Conjugation in Paramecium trichium Stokes (Protozoa, Ciliata) with special reference to the nuclear phenomena. Ralph Wichterman.

A cytological study was made of conjugation in a race of *Paramecium trichium* Stokes. The preconjugants, which are smaller than the vegetative individuals, fuse along their oral grooves. The centrally located oval macronucleus undergoes complete fragmentation involving the formation of a twisted ribbon which becomes thinner and longer resulting in small irregular rod-like elements. These result in spherical bodies which disappear in the cytoplasm after exconjugant reorganization.

The deeply staining oval-shaped micronucleus divides three times. The first pregamic division results in two micronuclear products; the second division, four products, three of which degenerate. The remaining micronuclear product enters into the third division to produce the pronuclei. The developing third pregamic spindle is commonly seen to press against the opposite conjugant where a cone is clearly visible. There is strong evidence to believe that the pronuclei cross approximately in the mid-region of the conjugants. This point is being investigated further by observing living conjugants under the microcompressor. Pronuclei fuse to form a synkaryon which divides three times to produce eight products. Two of these divisions occur while the conjugants are together; the third division occurs in the exconjugant. Exconjugants with four

macronuclear anlagen and a single micronucleus are commonly seen. This reduction in number from eight nuclear products of the synkaryon to five, is being studied further. Variations occur in regard to the distribution and fate of the eight nuclear products. Individuals with four macronuclear anlagen and one micronucleus divide with two anlagen going to each daughter while the micronucleus divides mitotically. In exconjugants with two anlagen and one micronucleus, a final division distributes a single macronuclear anlage to each daughter as the micronucleus again divides mitotically thus restoring the original nuclear condition to each animal.

This work confirms Diller's observations as reported in his abstract in *Anatomical Record,* 1934.

Mitotic activity of stimulated rat adrenals measured by colchicine technique. Opal M. Wolf.

In connection with a recent investigation it was noted that daily injections of an alkaline extract[2] of the anterior lobe of the pituitary into rats at 1 cc. and 2 cc. levels gave evidence of activity in the adrenals as measured by the colchicine technique. Control and injected animals were etherized the morning following the 2nd, 4th, 6th, and 10th days of injection. Nine to fifteen hours before the animals were killed, 0.3 mg. (per 100 grams of body weight) of colchicine [3] dissolved in distilled water was injected sub-cutaneously. At autopsy tissues were fixed in Bouin's, dehydrated by the aniline oil-dioxan method, cut at 10 micra and stained in Delafield's haematoxylin. Mitoses were counted with an oil immersion lens.

Preliminary observations were made of every 20th section in order to estimate the effect of colchicine on the organs. A 171 gram control male showed the following total counts:

Thyroid	157
Parathyroid	213
Adrenal	625 (highest count for controls)

An average count for the three largest sections of the adrenal, 45 in the controls, was used as a basis for comparison. Counts of both adrenals varied by 11–17 mitoses. Injected animals showed slight stimulation after 2 days, and greater stimulation after 4 days, but the differences were not marked for the two levels. The greatest stimulation occurred after 6 days of injection. One series of males weighing 170 grams gave:

Control	21
1 cc.	90
2 cc.	232 (2149—total for every 20 sections)

With one exception (126 mitoses on 2 cc. level), after 10 days injection the animals showed a count of 44. Nine to eleven hours after colchicine many of the cells were in early prophase, at 15 hours the greatest number were counted in the later stages. Most of the mitoses occurred in the outer part of the zona fasciculata, but a few cells in the zona glomerulosa and reticularis were dividing. No late stages of mitosis were observed in the medulla. In several instances very large nuclei appeared to be in early prophase.

[1] The experimental work was performed at Goucher College, the sections were cut and studied at the Marine Biological Laboratory.

[2] The growth extract was purchased from E. R. Squibb and Sons.

[3] Colchicine U.S.P. was purchased from Merck and Co. in the form of a powder.

The effects of the drug on the adrenal cells were similar to the descriptions by Ludford, Allen et al, and Nebel. Apparently normal stages of mitosis as well as stages leading to multi-nuclear cells were observed. The latter were probably caused by the varying concentration of the drug due to the rate of absorption and elimination. It was noted that the level of dosage had a toxic effect on some rats, others apparently showed no ill effects.

Induced breeding reactions in isolated male frogs, Rana pipiens Schreber.[1] Opal M. Wolf.

Adult male frogs give a characteristic call, show ridged epidermal thumb-pads with large, active mucous glands on the first finger of the fore limbs, develop amplexus, normally in the presence of the female, and shed sperms at the time of reproduction. In earlier work it was shown that the male as well as the female could be stimulated by the injection of frog anterior lobe to reproduce normally as early as the latter part of October and throughout the winter months. It seemed of interest to report experiments on individually isolated mature male *Rana pipiens*.

In the first experiments injections of frog anterior lobes from *Rana pipiens*, an extract of whole pig pituitary, a purified extract of the gonad-stimulating fraction of horse anterior lobe[2] and an extract of pregnant mare's serum, at times other than the breeding season and the summer, caused the shedding of sperm. Inspection of the cross-section of the testes showed empty seminiferous tubules and were comparable to the picture of the testes of frogs which were known to have fertilized eggs following pituitary stimulation. Following injection of the extract of pregnant mare's serum sperm were recovered as they passed from the red and swollen cloacal opening and stained preparations were made. In some cases the characteristic call or trill was heard 8 to 9 hours following the injection of as small an amount as one frog anterior lobe. The call was not heard following the injection of the extracts. The thumb-pads showed active mucous glands but the effect on the epidermal ridges was not so clearly shown because the thumb-pad of the adult male is ridged during the winter season.

Examination of the thumb-pads of adult males in the middle of July showed a smooth surface with slight indication of ridging and small mucous glands. A small piece from the thumb-pad of the experimental animals was removed as a control and the individually isolated animals were injected daily for 9 days with the anterior lobe from male *Rana catesbiana*. The injected animals gave the characteristic call of the breeding season, sperm were shed, active spermatogenesis was going on, the thumb-pads had developed ridges and the mucous glands were active. The thumb-pads of the experimental animals resembled those prepared from male *Rana clamitans* caught in the field and prepared immediately for histological examination. This animal breeds during the summer from June to the middle of August while *Rana pipiens* breeds from the first of April to the middle of May depending on the season and latitude.

It appears from an examination of the testes of the isolated experimental animals killed at varying times after prolonged pituitary treatment that the mature sperm are shed and spermatogenesis induced by frog anterior lobe and the extracts used in these experiments.

[1] Part of the experimental work was performed at the University of Wisconsin Department of Zoology and part at the Marine Biological Laboratory, 1936.

[2] The extract of horse anterior lobe, Prephysin A, prepared by Chappel Bros., and the other extracts prepared in Dr. Hisaw's laboratory were obtained through the courtesy of Dr. Frederick L. Hisaw.